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# PARASITOLOGY

## A SUPPLEMENT TO THE JOURNAL OF HYGIENE

EDITED BY

GEORGE H. F. NUTTALL, F.R.S.

Quick Professor of Biology in the University of Cambridge

AND

A. E. SHIPLEY, F.R.S.

Reader in Zoology in the University of Cambridge

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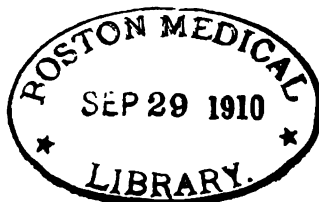
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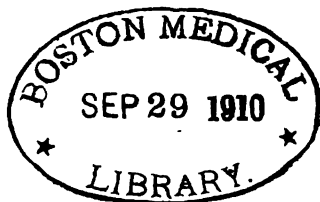


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## REVISION OF THE NON-COMBED EYED SIPHONAPTERA.

BY KARL JORDAN, PH.D., AND  
THE HON. N. C. ROTHSCHILD, M.A., F.L.S.

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### INTRODUCTION.

The importance of certain species of fleas in relation to the epidemiology of plague has been so amply demonstrated (see *Journal of Hygiene*, vols. VI. 1906, p. 426 and VII. 1907, p. 395) that an explanation appears scarcely necessary for our publishing a paper dealing with a revision of the non-pectinated eyed Siphonaptera. With the demonstration that fleas are capable of transmitting plague, their study at once ceased to be the mere hobby of a small group of entomological specialists. An accurate knowledge of fleas, both with regard to their structure and biology, is at present a matter of prime importance to those who concern themselves with the prophylaxis of plague.

The assumption seems justified that the species which is most abundant on rats in those portions of the world where plague is endemic will be the one which is most instrumental in spreading the disease.

In a paper by Rothschild (1906, p. 483<sup>1</sup>) it was stated that the species of flea usually found on rats in tropical and subtropical countries is *Pulex cheopis* Rothschild. When investigating the fleas which occur on any given host it appears to us to be essential to ascertain if there is more than one species associated with the spread of disease in the host, and to demonstrate precisely, in order to avoid confusion, the characters by which the various allied species can be distinguished.

It is obvious that false conclusions will be drawn by investigators who maintain that numerous similar (though in fact specifically distinct) forms are identical. Such observers have usually examined their specimens with eyes untrained for the special line of investigation upon which they have entered, with the result that they deny the existence of differences which are obvious to the specialist.

We shall, therefore, before describing the various species of *Loemopsylla* with which we are acquainted, point out the reasons which guide us in the discrimination of the differences which we consider to be specific and those which we consider to be non-specific.

The criterion of a "species" is a biological one. The fauna of a district consists of a certain number of units, each producing its own kind only; each being independent of the other in that respect; their own organisation only keeping these units apart. Such a unit we call the "species." Each species varies more or less and all individuals composing a species are different from one another. Again a species may be split up into well marked varieties, sometimes with and sometimes without the existence of intergradations. All individual varieties are, nevertheless, specifically identical, standing either in the relation of brothers and sisters or parents and offspring. It is for the specialist to determine in each group which forms are mere varieties of one unit and which forms are species. The ultima ratio is breeding. In a group where breeding from the egg has been carried out, at least in some instances, the systematist knows which differences are specific in these cases and which are not; and it is perfectly legitimate—in fact the only possible course that can be adopted—to determine by analogy the taxonomic value of differences observed in other allied forms. In the case of Siphonaptera we are at all events certain as to the specific distinctness of a number of common species. The flea of the domestic fowl, that of the domestic pigeon, the one from the house martin, and the flea from the sand martin, though formerly considered to be

<sup>1</sup> For literature relating to the transmission of plague by fleas see *Journal of Hygiene*, vol. vi. p. 433 (1906).

identical are now recognised as being perfectly independent of each other. The nests of the sand martin yield no other flea than *Ceratophyllus styx*, while henhouses are infested by *Ceratophyllus gallinae*, which are easy to rear. Rabbit warrens contain quantities of *Spilopsyllus cuniculi*, the insect being sometimes present in very large numbers on a rabbit, the individuals varying to a slight extent. From a careful comparative study of a very large series of specimens of such species, we arrive at a knowledge of the approximate limits of variation. The range of variation not being the same in the various organs, the most important point to ascertain is which organs are variable and which are comparatively constant in such undoubtedly distinct species. The characters here observed to be specific are a guide and a basis of classification in the case of allied forms about the life history of which nothing is at present known.

In the present treatise we are dealing with Pulicidae (*Loemopsylla cheopis* being one of them) which can be recognised by the following characters: the complete absence of a comb (= pecten or ctenidium) from the genal edge of the head as well as from the pronotum, and the presence of a strongly developed eye.

This group of fleas comprises six genera already recognised, namely, *Pulex* L., *Lycopsylla* Rothsch., *Moeopsylla* Rothsch., *Parapsyllus* Enderl., *Rhopalopsyllus* Baker, and *Goniopsyllus* Baker. We are, however, convinced that the division of this group into six genera only is an unnatural grouping. In the present article therefore a classification of these insects based upon a comparison of nearly all the known species has been attempted. This we think is especially desirable as considerable confusion appears to exist regarding the generic position of some of the species. The distinctions between the various species, too, do not appear to us to have been realized by all of those who have studied them. A concise exposition therefore of the generic and specific characters should, we think, be of use to those who have material they wish to name. It should, however, be clearly understood that the genera treated of in this paper are not more closely allied to each other than to some other Siphonaptera which have ctenidia. For instance, *Ctenocephalus erinacei* and *Spilopsyllus cuniculi*, which we exclude from this paper, are more closely allied to *Pulex irritans* than are the members of the American genus *Rhopalopsyllus*, which we shall deal with.

We intended at first to restrict this paper to an exposition of the species closely allied to *Loemopsylla cheopis* (which plays so prominent

a part in relation to plague), but on reflection we thought it would render the paper more useful, if we included also all the other Siphonaptera which have eyes but no comb on the head and pronotum. The scope of this paper has in consequence been considerably widened, but nevertheless we shall give most attention to the genus *Loemopsylla*.

**A synopsis of the genera to be discussed in  
the following pages.**

(I) Club of the antennae short, distinctly segmented only on the posterior side (Pl. II, fig. 1, 5). Antennal groove closed behind, the genal process separating the antennal groove from the forecoxa (Pl. II, fig. 1, 5, 13). Labial palpus consisting of four segments; tip of rostrum asymmetrical (Pl. II, fig. 8). Hindcoxa with a comb of short spines on the inner side (Pl. I). Fifth tarsal segment with 4 lateral bristles besides the subapical hair (Pl. III, fig. 7). Antepygidial bristle separate from the apical edge of the seventh tergite (Pl. V, fig. 5).

The species which belong to this group are all inhabitants of the Old World, but *Loemopsylla cheopis* has become more or less cosmopolitan, as the range of its hosts—the domestic rats—has increased.

We divide the species into four genera :—

1. Mesosternite very narrow, without internal rod-like incrassation from the insertion of the coxa upwards.....1. Genus: **Pulex**.

2. Frons without tubercle; anterior angle of genal edge produced backwards into a triangular lobe; pronotum longer than metanotum (Pl. II, fig. 1).

2. Genus: **Pariodontis**.

3. Frons with small mesial tubercle near the suture which separates the frons from the occiput; anterior angle of genal edge of head produced downwards into a triangular lobe; pronotum shorter than metanotum (Pl. II, fig. 13).

3. Genus: **Moeopsylla**.

4. Frons without tubercle; anterior angle of genal edge not produced into a triangular lobe; mesosternite with a rod-like internal incrassation from the insertion of the coxa upwards (Pl. I).....4. Genus: **Loemopsylla**.

(II) Club of antenna segmented all round, and the antennal groove open behind, the genal process being short (Pl. II, fig. 3, 12). Hindcoxa without a row of short spines on the inside. Labial palpus sharply segmented all round, consisting of 4 to 7 segments, the tip of the last segment being symmetrical (Pl. II, fig. 9). Fifth tarsal segment with 4 lateral bristles, besides a subapical hair. One antepygidial bristle, standing at the edge of the segment, which is sinuate (Pl. VI, fig. 7).

The species are American, with the exception of one, which has been found far south on an island in the Indian Ocean and on an island off the coast of West Australia.

Two genera :—

5. Club of antenna not symmetrical, the proximal segments sloping backwards (Pl. II, fig. 3). Genal process with only one or two bristles.

5. Genus: **Rhopalopsyllus**.



6. Club of antenna symmetrical, the proximal segments not detached on the hinder side and not sloping backwards (Pl. II, fig. 12). Genal process with a number of bristles. ....6. Genus: **Parapsyllus**.

(III) Club of antenna segmented all round, symmetrical (Pl. II, fig. 4). Hind-coxa without comb. Fifth tarsal segment with at least 5 lateral bristles, besides the subapical hair (Pl. IV, fig. 2).

The three genera which come under this heading are not at all closely related to one another.

7. Antennal groove open behind, the genal process being short and broad (Pl. II, fig. 4). Abdominal tergites with one row of bristles, except the first tergite, which bears two. Claws of tarsi with basal tooth; first midtarsal segment shorter than second. Two receptacula seminis in ♀ .....7. Genus: **Coptopsylla**.

8. Antennal groove open behind. Abdominal tergites with very numerous short bristles besides the long postmedian ones. First midtarsal segment longer than the second. ....8. Genus: **Goniopsyllus**.

9. Antennal groove closed behind. Abdominal tergites with one row of bristles, there being a few additional bristles on the first tergite. Claws of tarsi without distinct basal tooth. Antepygial bristle absent in both sexes. No stylet in ♀.

9. Genus: **Lycopsylla**.

#### 1. Genus: **Pulex** Linnaeus (1758).

*Pulex*, Linnaeus (1758, p. 614, partim, type of name: *irritans*); Geoffroy (1762, p. 615, partim); Fabricius (1775, p. 732, partim); Barbut (1781, p. 329, t. 18, fig. 5, copy of Geoffroy's fig.); Schellenberg (1798, p. 45); Latreille (1809, p. 366, n. 543, partim); Wood (1821, p. 124, partim); Swainson and Shuckard (1840, p. 393, partim); Bouché (1832, p. 503); Newman (1851, p. 143); Haliday (1856, p. 9, t. 1); Kolenati (1859, p. 65); Bielet (1881, p. 6); Boden (1882, p. 70, *Pulex* feeding on larva of a Tineid); Tyrrell (1884, p. 86); Dimmock (1884, p. 186, fleas eaten by earwigs); Blathwayt (1895, p. 345, t. 16); Wagner (1898, pp. 555, 575, partim); Baker (1904, p. 378, partim); Baker (1905, p. 128, partim).

**Head.** Frons without notch. Antennal groove closed behind, the genal process reaching almost to the lower posterior angle of the occiput. There is a strong internal incassation from the base of the antennal groove to vertex. The second segment of antenna transverse, bearing several long bristles at the apical edge; club short, round, solid on anterior side, the segmentation being barely indicated on this side, while the first three segments are deeply separated on the hinder side. There are a few very small hairs on the anterior side. Eye large, a little pointed below. Two bristles beneath the eye and a third at the oral edge. The anterior angle of the genal edge projecting somewhat downwards, and usually bearing a small tooth. Mandibles broad, short, and densely serrate. Rostrum shorter than the maxillary palpus,

reaching about halfway down the forecoxa. The labial palpus consists of 4 segments.

**Thorax.** Thoracic tergites short, each with one row of bristles; no subapical spines on the mesonotum. Prosternum widest close before apex. Mesosternite characteristic, very narrow, its ventral edge strongly oblique, the stigma not being entirely covered; no internal rod-like or cariniform incrassation from the insertion of the coxa to the dorsal edge. Episternum of the metathorax not quite separated from the sternum, the suture being indicated anteriorly only by an internal incrassation.

**Abdomen.** Convex in both sexes, dorsally as well as ventrally. The first tergite with two rows of bristles, the other tergites with one; the seventh with one long bristle a little before the apical edge. Stigmata large.

**Legs.** Midcoxa narrow, the internal rod-like incrassation dividing near base. Hindcoxa pear-shaped, long, being widest near base, hairy in front and behind on inner side, and bearing a row or patch of short spines near apex. First fore- and mid-tarsal segment shorter than second.

**Modified segments.** ♂: 8th tergite with small manubrium as in *Echidnophaga*. Clasper bearing a very large flap, on the inside of which there are two processes forming a kind of claw as in the *Sarcopsyllidae*; manubrium of clasper large, curved. Ninth sternite boomerang-shaped, its upper inner end pointed. Internal wire-like spring of ninth sternite and penis making several coils.—♀: no hairs above the stigma of 8th tergite. Stylet with long apical bristle and a short bristle before apex. Anal sternite truncate, the bristles confined to the apical edge.

This genus contains one species only, parasitic on man. In many respects it is the most specialized of all the *Pulicidae* and resembles in some characters the *Sarcopsyllidae*. The chief character of this insect is the greatly reduced thorax, the mesosternite especially being highly specialized. The pleura of this sternite is narrow and strongly oblique and lacks the internal cariniform incrassation found in other fleas. This incrassation usually extends from the suture of the coxa upwards to the anterior corner of the pleura, but it is absent from *P. irritans* and from some of the *Sarcopsyllidae*. In compensation for this loss the anterior ventral portion of the mesosternite (i.e. the sternum) is much strengthened inside. The peculiar structure of the ♂ genitalia separates *irritans* from all allied genera and links it with the *Sarco-*

*psyllidae*. The three free processes of the clasper are found in several of the old-world genera of the *Pulicidae*, the main characteristic, however, exhibited by *P. irritans* and the *Sarcopsyllidae*, i.e. that the second and third processes form a kind of claw, is not found in any of the other species. The genitalia of *P. irritans* are probably of a more primitive type than the genitalia of any other Siphonaptera (see Jordan and Rothschild, 1906, p. 38). *Pulex irritans* exhibits a further distinguishing character in the position of the bristles on the head, the bristle situated in front of the eye in most species being absent in *irritans* and replaced by one below the eye, as is also the case in *Loemopsylla chephrenis*. The hindcoxa—as is the case in some species of the genus *Loemopsylla*—is pear-shaped, but in *Pulex irritans* this coxa can be distinguished from that of any other known flea by bearing a number of hairs situated on the inner surface of the posterior (= meral) portion. A most interesting feature in the morphology of this flea is the presence in a large proportion of specimens of both sexes of a small tooth at the genal edge of the head, slightly behind the lower oral corner. This tooth from its position and structure corresponds to the fifth tooth of the genal comb of *Ctenocephalus canis* and allies. This single small tooth of *P. irritans* (often absent) is the last remnant of the genal comb with which, we think, the ancestral forms of *irritans* were provided. In the case of *Ctenocephalus erinacei* the teeth of both the genal and the prothoracic combs are small in size and few in number and occasionally almost disappear. This fact seems to us to be further evidence that the ancestral forms of *P. irritans* possessed both genal and prothoracic combs.

### 1. *Pulex irritans* Linnaeus (1758).

*Pulex*, *Puce*, *Floh*, *Flea*, etc., Moschetti (1544); Schwenckfeld (1603, p. 550); Hooke (1665, p. 61, t. 32, ♀); Borrich (1676, p. 185); Charleton (1677, p. 53); Muralto (1682, p. 137); Griendel von Ach (1687, p. 17, fig. 4); Bonanni (1691, fig. 56); Leeuwenhoek (1695, p. 20); Leeuwenhoek (1698, p. 325, fig. 1-20); Cestone (1699, p. 42, fig.); Leeuwenhoek (1706, p. 2311, proboscis); Bonanni (1709, p. 345, t. 377, fig. 49, t. 378, fig. 50-56); Camerarius (1714, p. 71); Joblot (1718, t. 13, fig. 6); Vallisnieri (1733, t. 25, fig. 1); Frisch (1734, p. 8); Cuno (1734); Bertolotto (1834); Albin (1736, p. 69, t. 41, ♀); Linnaeus (1744, p. 96); Adams (1743-46, t. 27); Rösel (1749, p. 10, t. 4, fig. 26, ♂, partim, good descr. of life-hist. of dog-flea); Linnaeus (1748, p. 67); Baker (1753, t. 13, fig. 6); Linnaeus (1756, p. 73); Kniphof (1759, § 15); Ledermüller (1761, p. 41, t. 20, ♀, metamorphosis); Geoffroy (1762, p. 616, t. 20, fig. 4, ♀); Weiss (1762, p. 340); Yeats (1773, p. 243); Degeer (1778, p. 7, t. 1, fig. 1); Martynn (1785); Ray (1788, p. 483); Latreille (1796, p. 172); Fitzgerald (18—?, p. 268,

fig.) ; Hadfield (1867, p. 837 ; on sandy shore, South India, in great abundance ; this species ?).

*Pulex vulgaris* Raius (1710, p. 7) ; Linnaeus (1730, p. 78).

*Pulex ater* Linnaeus (1746, p. 342, n. 1171).

*Pulex irritans* Linnaeus (1758, p. 614, n. 1, partim) ; Linnaeus (1760, p. 614, n. 1, partim) ; Sulzer (1761, p. 62, n. 65, t. 22, fig. 146 f) ; Linnaeus (1761, p. 479, n. 1965, partim) ; Scopoli (1763, p. 386, n. 1055, partim) ; Linnaeus (1768, p. 1021, n. 1, partim) ; Bourgeois (1769) ; Fuessly (1775, p. 59, n. 1175) ; Müller (1775, p. 1040, partim) ; Fabricius (1775, p. 732, n. 1, partim) ; Sulzer (1776, p. 242, t. 29, fig. 6 e, partim) ; Müller (1776, p. 182, n. 2208) ; Schrank (1781, p. 509, n. 1040, partim) ; Fabricius (1781, p. 381, n. 1, partim) ; Fabricius (1787, p. 314, n. 1, partim) ; Amoreux (1789, pp. 103 and 268) ; Roemer (1789, p. 33, n. 5, t. 39, fig. 6 e) ; Villiers (1789, p. 42, n. 1, partim, literature) ; Gmelin (1790, p. 2923, n. 1, partim) ; Fabricius (1794, p. 209, n. 1, partim) ; Shaw and Nodder (1794, t. 178, text nec figures) ; Jördens (1801, p. 41, t. 6, fig. 17, 21, 29, partim, literature) ; Sibly (1802, p. 431, partim, general account of structure and habits) ; Stewenson (1802, p. 232, partim) ; Walckenaer (1802, p. 353, n. 1, partim) ; Schrank (1804, p. 194, n. 2630, partim) ; Shaw (1806, p. 456, t. 122, ♂ nec ♀, partim) ; Wilhelm (1811, p. 304, t. 38, partim) ; Leach (1815, p. 126, "there are a vast number of species which have been confounded with *P. irritans*") ; Lamarck (1816, p. 334, partim) ; Savigny (1816, p. 27) ; Samouelle (1819, p. 234) ; DeFrance (1824, p. 440, partim, metamorphosis) ; Guérin (1825, p. 244) ; Kirby and Spence (1826, p. 471, t. 7, fig. 8) ; Stephens (1829, p. 328) ; Bouché (1835, p. 147, t. 4, fig. 1) ; Thon and Reichenbach (1838, p. 469, t. 131, fig. 1 a-r, ♂, ♀, metamorphosis) ; Lucas (1839, p. 393, t. 621, fig. 7) ; Blanchard (1840, p. 633, n. 1) ; Westwood (1840, p. 492) ; Dujardin (1843, t. 15) ; Gervais (1844, p. 365) ; Lucas (1849, p. 624) ; Agassiz (1855, p. 414) ; Walker (1856, p. 2, n. 1) ; Maitland (1857, p. 310) ; Dallas (1857, p. 381, fig.) ; Kolenati (1859, p. 65) ; Kolenati (1863, p. 31, n. 4, t. 1, fig. 2) ; Schenkling (1864, p. 693) ; Streubel (1866) ; Barton (1866, p. 316, in great abundance at Ventnor) ; Dufour (1861, p. 255, cocoon) ; Furlonge (1870, p. 189) ; Cooke (1871, p. 98) ; Furlonge (1872, p. 12) ; Ritsema (1873, p. 94, Holland) ; Ritsema (1874, p. 76) ; Wood (1876, p. 594, fig. 69, 2) ; Taschenberg (1880, p. 64, n. 4, t. 1, fig. 4) ; Ritsema (1881, p. 81, Holland, also on cat) ; Scott (1882, p. 9) ; Kraepelin (1884) ; Bergh (1885, pp. 1-6, fig. 25-29, larvae on skin of a woman) ; Packard (1889, p. 389) ; Raillet (1890) ; Smith (1894, p. 38) ; Packard (1894, p. 330, fig. 16) ; Baker (1895, pp. 65 and 67) ; Anonymous (1895, p. 142) ; Perez (1895, p. 238) ; Meinert (1896, p. 182) ; Osborn (1896, p. 147, fig. 80) ; Jourdain (1899, p. 204, "syringostome") ; Sharp (1899, p. 525) ; Oudemans (1900, p. 596, fig. 344, 1) ; Hilger (1901) ; Froggatt (1901, p. 539, fig. A, ♀) ; Wagner (1902, p. 129) ; Wahlgren (1903, p. 185, Australia, Tenerife, Sweden) ; Kohaut (1903, p. 33, t. 3, fig. 1-5, Hungary) ; Tiraboschi (1904, p. 246, fig. A, ♀, fig. 14, ♂) ; Baker (1904, p. 379) ; Baker (1905a, p. 129) ; Meissner (1905, p. 68) ; Jordan and Rothschild (1906, fig. B, E) ; Tiraboschi (1907, p. 580).

*pulex irritans* Latreille (1802, p. 577, t. 12, fig. 1, 2) ; Latreille (1805, p. 411).

*Pulex hominis* Dugès (1832, p. 163).

*Pulex simulans* Baker (1895, pp. 65 and 67, Texas, off *Didelphys virginiana*); Webster (1904, p. 244).

*Pulex irritans*, var. *dugesi* Baker (1899, p. 37, Mexico, off *Spermophilus*).

*Pulex irritans* var. *simulans* Baker (1904, pp. 379 and 457, occurrence on *Didelphys* accidental).

*Pulex dugesi* Baker (1904, p. 379); Baker (1905, p. 129).

Linné recognised two species of fleas only, *Pulex irritans*, which he diagnosed as *P. proboscide corpore breviori*, in contradistinction to the "chigoe," *Pulex penetrans*, of which he says *P. proboscide corporis longitudine*. Linné's *P. irritans* comprised various very distinct species of flea, such as those found on the rabbit and dog, in addition to the human flea, the true *P. irritans*. Leach, Bouché, Taschenberg and other authors have properly restricted the name *irritans* to the flea parasitic on man, the names *vulgaris*, *ater* and *hominis* of Raius, Linné and Dugès being treated as synonyms. The nearest allies of this species which are found on various animals are all inhabitants of the Old World. The fleas found in America are only distantly related to *P. irritans*. It may therefore be concluded that the present species originated from the Old World stock of Siphonaptera. Our knowledge of the variation and the present and former distribution of this insect is extremely meagre. Nevertheless, there are some points to which attention may with advantage be drawn. The insect is practically cosmopolitan, or, rather, has become cosmopolitan; specimens identical with European *irritans* are found almost everywhere. This was probably not the case prior to the introduction of the systems of intercommunication between all parts of the globe such as have been created in more recent periods. The tropical countries of the eastern hemisphere do not appear to be inhabited by *P. irritans* except where European settlements exist, or, at least, where intercourse with Europeans goes on. Ports, apparently, are everywhere infested by this parasite. We have failed to find any definite records of fleas in books on travel published in the early part of the nineteenth century. Although travellers in the tropics of Africa and Asia frequently complain of the abundance of lice on the natives in their huts, yet fleas do not seem to have been observed.

If it is a fact, however, that the tropical countries of the Old World were originally devoid of *Pulex irritans*, the reasons for the absence of the insect in question are worth considering. Two causes, of a biological character, may conduce to the absence of a species from any particular district. On the one hand the conditions of life may not

have been suitable, while on the other the species may have been debarred from reaching the district by a mechanical barrier of some sort or another.

The absence of *Pulex irritans* from the oases of the Sahara and from the Haussa countries south of the Sahara appears to be a well-established fact. The German explorers Nachtigall and Rollfs definitely state that fleas are absent from these countries, their remarks being corroborated by Dr E. Hartert, who also failed to find fleas in the Haussa countries, though lice were plentiful. The presence of lice would demonstrate that the habits of the natives would permit fleas to thrive, and it is therefore not unlikely that the soil and climate are unsuitable to fleas in these regions of the earth. These countries, moreover, have for many years been in communication with localities where *Pulex irritans* is known to abound. On the other hand the climate and soil of some districts of the Ethiopian region appear admirably suited to the human flea. Anderson (1856, p. 20), when starting on his expedition in 1850 from Walfisch Bay to Lake N'gami, found "myriads" of fleas in a deserted house formerly inhabited by a trader and situated some three miles inland. This statement corroborates what other observers have recorded, namely, that the flea propagates in deserted dwellings, the adult insect not requiring food to enable it to reproduce its species, at least for some time. An observation of a similar character is recorded by Euting (1896, p. 11). On an expedition from Damascus inland, Euting visited a deserted village and found his clothes on leaving it literally covered with fleas. These fleas, he says, appeared in the deserted dark rooms of the stone buildings and were apparently benumbed and without strength from their long fast.

In North, South and East Africa where there are European settlements, *Pulex irritans* appears to thrive well, attacking not only Europeans but also the natives and wild and domesticated animals. Should this parasite have formerly been absent from these countries, this fact can only be explained by the assumption of the existence of some geographical barrier, or that those tribes of men who came from the north and penetrated southwards into Africa lacked the flea. In the Oriental Region similar phenomena to those stated exist in respect to the distribution of this insect. In those parts of the East where European colonies exist and where free intercourse between Europeans and Orientals takes place, *P. irritans* is well established and appears to thrive, but it is not known whether this was the case among purely native populations prior to the advent of European traders and settlers.



The division of mankind into various races, many of them as distinct as the various species of some genus among other mammals, would lead one to expect that a corresponding differentiation would have taken place among the fleas parasitic on them; and that the human flea would now consist of a number of different races each peculiar to its particular human host. Some development of this nature in fact appears to obtain in at least one instance. Dr Carl Baker (1899, p. 37) described a flea found by Dr Dugès in West Mexico (Guanajuato) on *Citellus macrourus*. This insect Dr Baker considered at first to be a variety of *Pulex irritans*, calling it *Pulex irritans* var. *dugesi*, but later he treated it as a distinct species. We have two examples of this *dugesi*, for which we are indebted to Dr Baker, and possess also both sexes of the same form taken off Mexican Indians at Tabasco, which we received from the late Dr Buller. The differences between these Mexican *dugesi* and the true *Pulex irritans* are slight, but, nevertheless, fairly constant. The specimens are smaller in size, the rostrum (labial palpi) is longer, and the large flap of the ♂ clasper is less rounded at the apex. From an examination of our series of *Pulex irritans* from various South American countries, we find that a number of ♀ examples found on *Conepatus* in Bolivia agree with *dugesi* in respect to the length of the rostrum, but are not inferior in size to the true *irritans*. Other examples from Peru, Chile, Argentina, Paraguay and Brazil, taken in houses and on various mammals, do not differ from typical European *irritans*. The occurrence of a slightly different race of *Pulex irritans* in Mexico and Bolivia, and possibly in the intermediate countries, both on the natives and on mammals, raises two interesting points, first, whether *Pulex irritans* originated as a flea found on mammals and then adopted man as its host, or if it developed to what it now is on becoming a parasite of man. In Europe and Central Asia this flea is essentially a parasite of man, occurring only occasionally on other warm-blooded creatures. The same may be the case in other countries, the frequent occurrence of this insect on mammals in America being possibly explained by the closer connection between human dwellings and wild and semi-domesticated beasts. Dirty and deserted huts frequented by small mammals may also induce the human flea to propagate more freely. As we have pointed out the imagines of the human flea can exist a long time without food, while the larvae, as opposed to being parasitic, feed on all kinds of dirt. In respect to the second point mentioned above, namely, whether the present species has undergone modifications since becoming a parasite

of the human race, many speculations could be indulged in. If the "Indians" inhabiting Mexico (and presumably those of other countries of South and Central America) have a special race of *Pulex irritans*, it is obvious that the parasite has either developed into this special race after the Indians came to America, or that they were from the beginning infested by this special race of *Pulex irritans* and not by the ordinary form of *P. irritans*, when they came to America, possibly from Asia. If the latter surmise should be correct then this race of flea may still exist in the original country whence these Indians came.

The individual variation in the specimens of *Pulex irritans* is slight and refers especially to the number of bristles situated on the sclerites of the thorax and on the legs. The most prominent variation is that of the bristles of the femora and of the short spines on the inner side of the hindcoxa. These spines are sometimes but few in number, while again they may be numerous, the variation in respect to the number of these spines in our series of mounted specimens being from 6 to 14.

We have specimens of *Pulex irritans* from various places in Europe, off man and badger<sup>1</sup>; Malcoci, Rumania, off fox and *Putorius putorius*; Rio de Oro, N. W. Africa, off *Gerbillus riggenbachi*; Mogador, Morocco, off *Vulpes niloticus*; Hadgine, Taurus, off dog; Adana, Asia Minor, off cat and *Canis aureus*.

Cairo, Egypt, off *Canis zerda* and *Erinaceus auritus*; Akaki, Abyssinia, off man; Ginir, Abyssinia, off *Canis* spec.; Berbera, N. E. Africa, off dog; Dairoli, Abyssinia, off dog; Sidimun, Somaliland, off *Herpestes gracilis*; Deelfontein, Cape Colony, off *Felis caracal* and *Tinamus* spec.; Kingwilliamstown; Benguella, Angola, off *Canis* spec.; Island of St Thomé, Bay of Benin, off a sea-bird.

Yokohama, off dog, cat, and in a house; Kiu-Shiu, Japan, off man; Bombay, off man; Lhassia, Assam, off dog; Gippsland, Victoria, off *Echidna hystrix*; Paramatta, N. S. Wales, off man; Western Australia.

Alberta, Canada, off *Lynx* and *Vulpes velox*; Frontera, Tabasco, Mexico (= *dugesii* Baker), off natives; Belize, British Honduras, off man; Peru, in houses; Pampa Olliga, Bolivia, off *Conipates arequipae*; Choro, Bolivia, off *Conipates churensis*; Sapucay, Paraguay; Temuco, Chile, off man and dog; Valparaiso, Chile, in a house, in sand, and off *Canis griseus*.

<sup>1</sup> In England *P. irritans* is an undoubted parasite of the badger (*Meles taxus*), from freshly captured wild examples of which animal taken near Reading and Hastings we have secured series of this insect. *P. irritans* occurs occasionally on rats and mice in houses and ships.

2. Genus: *Parodontis* gen. nov.

**Head.** Frons without tubercle (Pl. II, fig. 1). Genal edge produced at anterior angle into a long curved tooth. Antennal cavity closed. Second segment of antenna transverse, bearing a row of long hairs, which reach beyond the club in ♀; club globular, a little longer in ♂ than in ♀, anteriorly solid, but the lines of separation of the central and distal segments distinctly marked, especially in ♂; proximal segments of club free on hinder side. Internal incassation from base of antennal groove upwards not very distinct in ♀. Eye round; usually a small bristle in front of it, a second beneath it, and a third above oral edge. Mandible rather broad, densely serrate at apex. Labial palpus consisting of 4 segments.

**Thorax.** Tergites with one row of bristles; no spines before apex of mesonotum. Pronotum longer than mesonotum (Pl. II, fig. 1), and this longer than metanotum. Epimerum of mesothorax oblique, the stigma being partially uncovered. Episternum of metathorax large, separated from the sternum. Bristles of thorax and abdomen stout. Prosternum widest before apex.

**Abdomen.** Tergites with one row of bristles; seventh tergite bearing one long bristle before the apical edge. Stigmata large.

**Legs.** Internal rod-like incassation of midcoxa dividing above the centre. Hindcoxa with comb of short spines on inner side. Ventral surface of hindfemur evenly convex. First fore- and midtarsal segment shorter than the second. Fifth tarsal segment considerably dilated towards apex.

**Modified segments.** Modified segments of the same type as in *Loemopsylla*.

This genus also, like *Pulex*, contains but one species, which is found apparently all over Africa on *Hystrix cristatus*.

The genus *Parodontis* is undoubtedly allied to *Pulex* and *Loemopsylla*. *Parodontis riggenbachi*, however, is so distinct from *irritans* and *cheopis* that it demands a special genus.

In the non-pectinated eyed *Pulicidae*, with the exception of the present genus, all three thoracic tergites, or at least the pro- and mesonotum, are short, and show a distinct tendency to become more and more reduced in length as specialisation continues.

In *P. riggenbachi*, on the other hand, the prothorax is longer than in any other member of the family *Pulicidae*, and the mesonotum also is not reduced. The long thorax may be an ancestral character, but

the pronotum possibly has secondarily become longer than it originally was.

*Pariodontis* exhibits the extreme development as regards the extension of the tergites of the thorax, while the *Sarcopsyllidae*, on the other hand, have the tergites of the thorax reduced to narrow strips, *P. irritans* and *L. cheopis* (and allies) representing intermediate phyletic stages.

The *Sarcopsyllidae*, though differing so markedly from *Pariodontis* in the development of the tergites of the thorax, have a character which is not found in *Pulex* and its other near allies, but is present in the porcupine flea, this being the curved tooth (projecting back- and downwards) into which the lower oral corner of the mouth is prolonged. This hook is present in all the *Sarcopsyllidae*, but in the other families is absent, except in the genera *Pariodontis*, *Moeopsylla* and *Lycopsylla*. This character has probably been acquired independently in these different genera, serving the same purpose as the genal comb of other fleas, namely, to prevent hairs getting into the joint between the head and the prosternite when the parasite is passing through the fur of its host. In other respects *P. riggenbachi* is closely allied to the genus *Pulex*, as well as to *Loemopsylla*, but resembles *Loemopsylla* in the structure of the male genitalia. The male genitalia of *Pariodontis* in fact show the same specialised form as in *Loemopsylla* and differ markedly from the male genitalia of *P. irritans*.

(1) *Pariodontis riggenbachi* Rothsch. (1904).

(Pl. II, fig. 1.)

♂ ♀, *Pulex riggenbachi* Rothschild (1904, p. 611, n. 7, t. 8, figs. 19, 20, t. 9, fig. 24, Cape Colony and Morocco, name-type from Cape Colony).

The first specimens of this large species were sent to us by W. Rigenbach, who collected them off *Hystrix cristatus* in Morocco in 1900. Since then we have received the insect from other parts of Africa also, the host being in every instance the same.

Mogador, Morocco, November 1904, and Mazagan, Morocco, collected by W. Rigenbach.

Deelfontein, Cape Colony, July 1902; C. H. B. Grant.

Mangona R., Bihé, Angola, January 1904; Dr W. J. Ansorge.

3. *Genus: Moeopsylla* Rothsch. (1908).

*Moeopsylla* Rothschild (1908, p. 3).

Very closely allied to *Loemopsylla* and *Pariodontis*, but differs in the following characters:

**Head.** Frons with a minute tubercle situated close to the suture which separates the frons from the occiput. The genal edge dilated into a tooth as in the *Sarcopsyllidae*, *Lycopsylla*, and *Pariodontis* (Pl. II, fig. 13). The segmentation of the rostrum distinct only on the anterior side.

**Thorax.** Bristles of the pronotum nearer to the base than to the apex. Metanotum much longer than the mesonotum. Mesosternite narrow, the internal rod-like incrassation, which extends from the insertion of the coxa upwards, ending at the anterior margin of the sternite instead of at the dorsal margin (Pl. II, fig. 13).

**Legs.** Hindcoxa with a patch (not a row) of very numerous short spines on the inner surface. Basal tooth of the claws very small.

One species from Africa.

(1) *Moeopsylla sjoestedti* Rothsch. (1908).

(Pl. II, fig. 13.)

*Moeopsylla sjoestedti* Rothsch. (1908, p. 3, t. 1, figs. 1—4).

Professor Y. Sjoestedt found a small series of both sexes of this species on *Phacochoerus africanus* in the Massaisteppe, German East Africa, on the 9th October 1905.

4. *Genus: Loemopsylla* gen. nov.

**Head.** Frons without a notch or tubercle (Pl. I). Genal process almost completely closing the antennal groove, separating it from the prosternum and being pointed behind. Antenna different in the sexes, the first segment being long in the ♂ and short in the ♀; the second, transverse segment bearing a number of long bristles at the apical edge. The first segment of the club compressed and leaf-shaped; the globular or slightly elongate club appearing solid on the anterior side, the incisions between the segments being hardly at all indicated, while on the hinder side the first two or three segments are deeply separated from one another (Pl. II, fig. 5). On the anterior side of the club there are from two to five very short hairs. Antennal groove in ♀ not

extending to vertex, the internal incassation from the groove upwards being vestigial or absent. Eye round. One bristle beneath the eye, a second at the oral edge, and a third, often small, in front of the eye. Labial palpus consisting of four segments.

**Thorax.** One row of bristles on each tergite. On mesonotum no spines between the row of bristles and the apical edge. No small teeth at the edge of the metanotum. Epimerum of the mesothorax oblique, not completely covering the stigma, the suture between epimerum and sternum distinct. Prosterum widest behind (Pl. I).

**Abdomen.** Convex above and below in both sexes. No small teeth at the apical edges of the tergites, except the first, which bears sometimes two teeth. Seventh tergite with one long apical bristle either on a tubercle, placed away from the apical edge (Pl. VI, figs. 1—4), or, as only in ♂ of *L. scopulifer*, on a long cone (Pl. V, fig. 1).

**Legs.** Internal rod-like incassation of the midcoxa forked near the base. Hindcoxa always with comb of small spines on the inner side (Pl. I). Mid- and hind-femora with a row of bristles on the inner side. First fore- and midtarsal segment shorter than the second (Pl. III, figs. 4—6, 8).

**Modified segments.** ♂. Clasper with two or three small processes; manubrium narrow; upper internal portion of the ninth sternite not very sharply defined (Pl. IV, figs. 6—12).—♀. The stylet bearing, besides the long apical bristle, a short bristle situated in a notch before the apex.

**Distribution.** Africa and Central Asia, one species (*cheopis*) apparently in all warm countries, being distributed with rats.

Type of name: *cheopis* Rothsch.

Many of the species of this genus differ in size, but they all conform to one type in outline. The thorax being short and the abdomen convex above and below, the species of *Loemopsylla* are compact in aspect, resembling in this respect several other Pulicidae, for instance, *Spilopsyllus cuniculi*, *Rhopalopsyllus australis*, *Ctenocephalus erinacei*, and others. The characters however by which all the species of *Loemopsylla* are distinguished from other fleas are sufficiently trenchant, we think, to render it impossible not to recognise which species belong to this genus and which do not. The most obvious of these distinguishing characters of *Loemopsylla* are the four-segmented labial palpus, the closed antennal groove, the anteriorly solid antennal club, the division of the pleura of the mesosternite by a suture into a sternal and a meral sclerite, the position of the dorsal apical bristle of the seventh abdominal tergite remote



from the edge of the segment, the presence of short spines on the inner surface of the hindcoxa, the division of the rod-like incrassation inside the midcoxa taking place near the base, and the structure of the modified abdominal segments. By observing these characteristics, confusion with other genera is easily avoided.

The species are more numerous in *Loemopsylla* than in the allied genera, or it is perhaps safer to say, more species have become known. Most of these the junior author of the present paper collected himself in Egypt and the Egyptian Soudan. The genus is essentially African, being generally found on desert mammals, one of the species (*L. cheopis*) having become cosmopolitan with its hosts, while three others are only known from Central Asia. They are essentially fleas of rodents, *L. cheopis* being especially often found on rats. As fleas leave the host when it dies, and take to another host, they may become the bearers of germs and hence the means of the spread of infectious diseases, one of the rat-fleas, *L. cheopis*, being the carrier of the plague-germ from rat to rat and from rat to man, and another species, *L. cleopatrae*, also being the means of the spread of disease. A special interest therefore attaches to the study of these insects, and we have accordingly thought it desirable to give here a general outline of their morphology, before proceeding to state the chief characteristics by which the various species of *Loemopsylla* can be recognised. A comparison moreover of the morphological characters of *Loemopsylla* with the other fleas will show clearly the relationship in which this genus stands to other genera.

As we have said above, the compactness of the body is partly due to a reduction in length of the thoracical segments. This reduction has not spread to the head, the capsule of which is large when compared with the thorax. The head being the bearer of the piercing and sucking organs which require a supply of strong muscles, there must be room in the caputal capsule for these organs and their retractors and extensors. We can therefore hardly expect to find the head reduced to any great extent in the fleas which have well developed piercing organs. These organs, consisting of the upper lip (labrum)<sup>1</sup> and the two mandibles, are slenderer and longer in *Loemopsylla* than in *Pulex irritans*, agreeing on the whole with those of the American non-combed eyed fleas. The slender type of mandible is doubtless more ancestral than the broad and heavily serrate type. The latter obtains in all *Sarcopsyllidae* and *Spilopsyllus cuniculi*, as well as in *Pulex irritans*, and to a less extent

<sup>1</sup> According to Kraepelin and Heymons, whose opinion we believe to be correct, other authors call the organ epipharynx.

in *Ctenocephalus erinacei*. Now, the species with the broadest and most heavily serrate mandibles are stationary parasites, fixing themselves firmly in the skin of the host by means of the piercing organs. As the stationary mode of living is without doubt a secondary development, we may accept the broad mandibles to be the result of this change in habits, the mandibles (at least in these closely allied *Pulicidae*) having developed a new function, to assist the insect in retaining its hold as well as to pierce the skin. *Pulex irritans*, however, is a very active insect and by no means stationary.

The width of its mandibles and their strong serration therefore cannot be explained in the way indicated, unless we assume that the ancestral *P. irritans* was a comparatively stationary insect when it adopted man as host. This assumption, however, does not appear to be satisfactory, since it is hardly likely that a stationary parasite should become active again, there being no sufficient reason for this regression to a former state of habits, stationary ectoparasites thriving very well on man. We think, on the contrary, that the strong piercing organs of *P. irritans* were acquired after man had become the host of the insect, the naked skin and the garment covering it rendering the claws of the legs insufficient for keeping the insect steady when sucking, the strengthening of the mandibles, moreover, preventing them from being easily injured. The upper lip also is strong in *P. irritans* and the stationary fleas mentioned, bearing along the anterior edge a number of obtuse teeth. These teeth are present in all fleas, but are often very few in number, being restricted to the apex of the organ. The species of *Loemopsylla* agree with *Pulex irritans* in the armature of the upper lip, except that the teeth are less prominent and fewer in number, while the American non-combed eyed fleas have only two or three such teeth, placed near the apex of the upper lip.

The piercing organs, when at rest, are retracted and the external portion is encased in a tube (= rostrum) formed by the two labial palpi, which are situated at the apex of the short non-divided labium. The number of segments composing the labial palpus as far as we know varies in the *Siphonaptera* from 2 to 17. In most fleas, however, the labial palpus consists of five segments. This appears to be the original state of development, the palpus with more and the one with less segments being a derivation from the normal five-jointed type. The rostrum is not a piercing organ like that of bugs and flies. The two palpi separate, when the mandibles and upper lip, which are interlocked to form a tube, penetrate the skin of the host, lying flat right and left on the skin, or

protruding upwards when the head is embedded in it, as is the case in the *Sarcopsyllidae* (Jordan and Rothschild, 1906, p. 27). The labial palpi therefore require to be flexible and this is attained by segmentation or by a reduction in chitinization. The latter development obtains in the *Sarcopsyllidae*, all species of which family have a very pale, weak, soft rostrum. The necessary flexibility of a prolonged and strongly chitinized rostrum as it is found in various fleas (*Vermipsylla*, *Macropsylla*, etc.) is obtained by greater segmentation. The species of *Loemopsylla*, *Pulex* and *Pariodontis* and the combed fleas *Ctenocephalus*, *Hoplopsyllus*, *Spilopsyllus*, and a few forms provisionally placed in *Ceratophyllus*, as well as a number of eyeless fleas, have four segments in the labial palpus. All the American non-combed eyed *Pulicidae*, however, with one exception, have at least five segments in the labial palpus, the same being also the case in the Transcasian species *lamellifer* Wagn., which is in other respects also closely allied to the American forms. The reduction in the number of the segments of the rostrum is not always accompanied by a shortening of the organ. The rostrum of *Hoplopsyllus glacialis*, *Loemopsylla longispinus* and others, with four labial palpal segments, is longer than the rostrum of some fleas in which the labial palpus has five segments. The reduction does not take place from the distal end, the apical segment often remaining very long in the 4-segmented labial palpus. The reduction is not effected by the loss of a segment, but the disappearance of an incision, two segments being fused to form one. As the segments bear bristles at the apical edge, an intermediate stage of development between a 4- and a 5-segmented palpus would be a 4-segmented palpus with one of the segments bearing a bristle on each side in or near the centre. Such a labial palpus may exist, but we have not yet observed it. We think it likely that in the 4-segmented palpus the second segment has become fused with the first.

The bristles at the extreme tip of the rostrum are apparently sensory in character like those at the apex of the maxillary palpus. The insect is probably using them to test the skin of the host prior to puncturing it, at least so it appears when a hungry flea is placed on the hand or arm. These "testing" bristles of the rostrum differ both in number and size in the various groups of fleas; and as the variation within each group is but slight, they afford characteristics which are valuable for classification. There are apparently never more than six such bristles at the tip of each labial palpus, this being, it would seem, the normal or ancestral number, three bristles being placed on each side at the apical edge of the end-segment. This number is found in

the majority of fleas, the tip of the segment being more or less symmetrical (Pl. II, fig. 9). In other forms one or two bristles on one or both sides are lost, while in the *Sarcopsyllidae* all these bristles are absent. The non-combed fleas from the Old World, allied to *Pulex*, differ widely in respect to these bristles from those found in America. In *Rhopalosyllus* and the allied genera from America described hereafter the end of the rostrum is practically symmetrical with six bristles at the tip of each end-segment (Pl. II, fig. 9). These *Pulicidae*, therefore, have preserved the tip of the rostrum in a generalized state of development. On the other hand, in the Old World non-combed fleas, with the exception of *lamellifer*, the end-segment of each labial palpus is obliquely truncate, being asymmetrical and bearing only three bristles at the edge (Pl. II, figs. 8, 10, 11). The same very striking characteristic obtains also in *Otenocephalus* and *Spilopsyllus*, which are close allies of *Pulex* and *Loemopsylla*.

Some further peculiarities which separate the Old World allies of *Pulex* from the New World genera are the shape and structure of the prae-antennal or frontal portion of the caputal capsule. A large number of fleas possess the so-called frontal tubercle or notch in the centre of the frons, but nearer to the mouth than to the antenna. This tubercle is especially well developed in nearly all the species of *Ceratophyllus* and allied genera, being sometimes inserted in a groove. It attains its greatest development in *Listropsylla agrippinae* Rothsch. (1904 a, p. 634), and in this species is heart-shaped (or rather, like the "spade" in cards) and projects from a groove. The real nature of this organ is at present unknown, though presumably an organ of sense, and its homology is also uncertain. The organ is suggestive of the egg-breaker of the larva, but is probably a new acquirement, being perhaps neither a modification of some organ possessed by the ancestral Siphonaptera (*ocellus*, for instance), nor a remnant from the larval stage. The simple tubercle of many *Ceratophyllus* may not however be homologous to the tubercle placed in a groove, but in any case the organ is of considerable taxonomic value in those *Pulicidae* we are here dealing with. This tubercle situated in a groove is well developed in all the American forms, but is not met with in any species of the Old World genera *Pulex*, *Parodontis*, *Moeopsylla* and *Loemopsylla*. In *Moeopsylla* only there is a minute tubercle, which is not however situated in a groove and is placed much more dorsal than in the American species.

A second characteristic feature in the prae-antennal portion of the head of *Loemopsylla* and its allies obtains in the development of the

genal area, which is the portion of head situated beneath the eye and extending from the mouth to the antennal groove. This so-called genal process is in the various Siphonaptera either prolonged so far backwards as to meet the hind-edge of the post-antennal portion of the head and therefore closing the antennal groove behind, or it is short, being widely separated from the occipital (or posterior) portion of the head by the antennal groove, the latter therefore being open behind. The closed antennal groove (Pl. II, figs. 1, 5, 13) is found in *Pulex*, *Pariodontis*, *Moeopsylla* and *Loemopsylla* and a number of combed fleas, while the American non-combed allies of *Pulex* have the antennal groove open (Pl. II, figs. 3, 12), which is also the case in *Coptopsylla lamellifer* from Transcaspia (Pl. II, fig. 4). Two stages therefore in the phyletic development of the head can be observed in this group of fleas, and the question presents itself which of them is the earlier and which the later stage; or in other words, had the ancestors of these insects an open antennal groove or a closed one? From whatever order of insects the Siphonaptera may be a derivative, the antennal groove is a specialization and hence also the division of the head from the crown to the lower posterior corner. Further, the closed antennal groove is not merely a lateral impression surrounded by the solid skeleton of the caputal capsule, but the groove is closed, because the genal process of the prae-antennal portion of the head reaches to the post-antennal portion, there being a suture (or even a small gap underneath the genal process) which separates the apex of the genal process from the occiput. This suture (or gap) proves that there was here at one time a disconnection. The same conclusion is arrived at if we consider the antenna itself. The closed antennal groove of *Pulex*, *Loemopsylla* and allies corresponds to a reduction in the antennae. The *Pulicidae* with long and well-segmented antennae have also an open antennal groove extending on to the prosternite. The complete segmentation of the antennal club being regarded as an earlier phyletic stage than the short club, which is segmented on one side only, necessitates the conclusion that similarly the closed antennal groove is a derivation from the open groove, the closing of the groove being perhaps a consequence of the reduction in the size of the antenna.

The sexes of *Loemopsylla* differ considerably in the upward extension of the antennal groove, as they do in most other Siphonaptera. The groove reaches nearly to the vertex of the head in the ♂, there being, moreover, an internal incassation from the groove upwards, this being the suture, or the remnant of it, which in insects generally limits the

frons and is situated between the antennae. In the ♀ the groove does not extend nearly so far dorsad and the internal incassation is absent or vestigial only. The antennae themselves show a corresponding difference in the sexes, being longer in the ♂ than in the ♀, as is generally the case in fleas. The first segment especially is very much longer in the ♂ of *Loemopsylla* than in the ♀, the difference between the sexes being much slighter in the American forms. The second segment is transverse and bears in both sexes a row of long bristles at the apical edge. These bristles are longer in the ♀ than in the ♂. The club is somewhat shorter in the ♀ than in the ♂. The nine segments composing it are separated on the posterior side<sup>1</sup> by the segmental incisions, the incisions being especially deep between the first four segments. The anterior side of the club is solid, only three central incisions being faintly indicated. On the inner surface the club bears very numerous minute hairs in the ♂ only. The bristles of the head are likewise not quite the same in the sexes, inasmuch as there is in the ♂ along the hinder side of the antennal groove a row of small hairs, which is represented in the ♀ by a very few hairs only. These hairs<sup>2</sup> of the ♂ are not placed very close together, the interspace between every two being at least equal to one-fourth the length of the hairs. In this character the ♂♂ of *Loemopsylla* differ from the American *Pulicidae* of the present group of genera, the hairs being very close together in the ♂♂ of the American forms and also numerous in the ♀♀ (Pl. II, figs. 3, 12). The function of these short stiff hairs may be simply protective. The antenna is an organ of smell which plays an important sexual rôle, inasmuch as it enables the sexes to find each other, and might easily be injured if it were exposed<sup>3</sup>, when the flea is gliding through the fur or feathers of the host. The antenna is protected by being enclosed in the antennal groove, of which the anterior edge often partly projects over the groove. Possibly the row of short hairs at the posterior side of the groove may protect the antenna from behind. The hairs, however, may also serve as a kind of comb for cleaning the antenna, insects generally having some means or other (forelegs, mouth-parts) with which they are able to remove dust or dirt from this organ of sense. In *Rhopalopsyllus* they are placed on a carina (Pl. II, fig. 3).

The other bristles of the head are practically alike in the sexes.

<sup>1</sup> "Posterior" when the antenna is lying in the groove.

<sup>2</sup> Dahl (1898, p. 191) calls these bristles the remnants of a faceted eye.

<sup>3</sup> The antennae of ♂♂ when in copula are usually exposed; further observations on this point would be interesting.

They are, however, of some morphological interest and frequently offer characters of taxonomic value. The hinder portion of the head (or occiput) bears normally in fleas three rows of bristles, one near the base of the antenna, a second in the centre and a third near the hinder edge of the head. It is interesting to find that these three rows are continued also over the frontal portion of the head (Pl. II, fig. 3). Here the anterior row extends from the maxillary palpus to the base of the antenna, the second row is placed in front of the eye, consisting usually of three long bristles, while the third row, which is absent from most fleas, is situated on the genal process. The three rows divide the head in four sections, corresponding perhaps to the four segments of which the head is composed.

The thorax of the *Siphonaptera* has some very characteristic features. The three segments (pro-, meso- and metathorax) are each quite distinct, while the incision, however, between the pro- and mesothorax is deep, the meso- and metathorax are more closely applied to each other. The thorax therefore in this respect resembles most nearly that of *Coleoptera*, with this difference, that in *Siphonaptera* the hind edge of the mesonotum is similar to the metanotum and overlaps the same, and the metasternite projects farther ventrad than the mesosternite, the two segments not being so closely connected as in *Coleoptera* and *Rhynchota*. Each of the three thoracic tergites of *Siphonaptera* forms a simple half-ring, there being no distinct division—by a suture—into two principal sclerites, a scutellum and a post-scutellum, as in other holometabolous insects. In *Loemopsylla* the tergites are narrow and bear each only one single row of bristles, as is also the case in *Pulex*, *Otenocephalus*, and some other allied genera (Pl. I). In the ♂ of *Loemopsylla regis*, however, there are dorsally a few hairs in front of this row on the pro- and mesonotum. This single row corresponds to the postmedian row of bristles of other fleas, the bristles of this row being always longer than those of the preceding row or rows. The width of the segments of the thorax varies to a slight extent in *Loemopsylla*, the mesonotum especially being distinctly longer in some species than in others. The comparatively long mesonotum of the heavy-spined *Loemopsylla chaphrenis* Rothschild, and the likewise comparatively long metanotum of the long-bristled *Loemopsylla longispinus* Wagn., have only one row of bristles, while in some American allies of *Loemopsylla* these tergites, which have no greater width than in the *Loemopsylla* mentioned, bear two or even three rows of bristles. From this it is obvious that the

loss of bristles in *Loemopsylla* and the allied Old World genera is not a consequence of the reduction of the segments in width, but indicates rather the general tendency towards reduction obtaining in many organs of these Old World Siphonaptera. The pronotum of *Loemopsylla* never bears a comb, nor are there any slender, bristle-like, subapical spines on the mesonotum, nor has the metanotum ever a dentate or serrate apical edge.

The sternites of the thorax, with the exception of the prothorax, have preserved the original main division into an anterior and a posterior portion. The prosternite, as is very often the case in insects, does not show any distinct separation into several sclerites. It bears the coxae at its anterior corner, the whole prosternite therefore being postcoxal. The lateral portion of the prosternite, which is larger, especially in length, than the ventral portion, has in *Loemopsylla* the same shape as in *Pulex*, being widest near its hinder end. It is strongly chitinized beneath, being more or less flattened behind the coxae. The meso- and metasternites on the other hand are ventrally membranaceous from the insertion of the coxae backwards. The prosternite never bears any bristles in *Siphonaptera*, which is remarkable, since the meso- and metasternites have quite a number of bristles on the sides. Above the slanting posterior edge of the prosternite is the first stigma, situated between the prosternite and the protergite and concealed underneath the overlapping edges of these sclerites. In a mounted (cleared) specimen the circular trema of the stigma and the trachea are generally plainly visible (Pl. II, fig. 1).

The mesosternite exhibits a similar characteristic reduction in size and shape as in *Pulex*. There is, however, in *Loemopsylla*, as in the other *Pulicidae*, a rod-like internal incrassation laterally in the mesosternite, extending from the insertion of the coxa upwards and corresponding to the meral suture of other insects, which divides the sternite into an anterior (= sternal) and a posterior (= meral) portion (see diagram, figs. A and B). The meral suture itself is absent from the outer surface of the sternite in *Loemopsylla*. This internal rod-like incrassation is absent from *Pulex* and the *Sarcopsyllidae*. From the insertion of the coxa forwards there is another internal incrassation which corresponds to a suture separating in other insects the sternum from the episternum. The most anterior portion of the mesosternite of *Loemopsylla* is therefore homologous to the sternum, and the triangular portion situated further dorsal homologous to the episternum. The relative position of these two sclerites, which are entirely fused in



Siphonaptera, is best visible in the bat-fleas (*Ischnopsyllus*), in which the sternum is much longer than in the true *Pulicidae*. The posterior portion of the mesosternite, namely, the epimerum, has in *Loemopsylla*

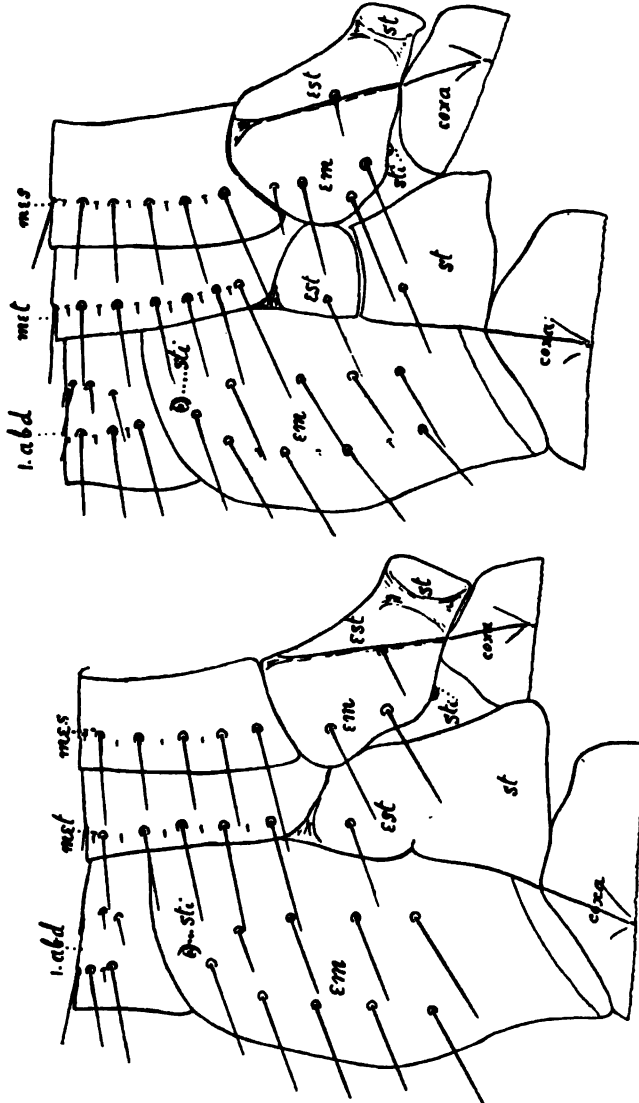


Fig. A.

Fig. A. Meso- and metathorax of *Loemopsylla pallidus*.

Fig. B.

Fig. B. The same of *Loemopsylla cheopis*.

mes = mesonotum ; met = metanotum ; st = sternum ; est = episternum ; em = epimerum ;  
sti = stigma ; 1.abd = first abdominal tergite.

the same shape as in *Pulex irritans*, being much smaller than in the New World non-combed *Pulicidae*. The ventral posterior edge of this epimerum recedes obliquely upwards, the stigma, which is situated

beneath it, projecting a little below the epimerum. This stigma, or rather the walls of the cavity in which the trachea opens, is nearly globular, appearing in a lateral view to be situated on a short stalk, the whole being cup-shaped (Pl. II, fig. 1, sti). This organ was formerly often mistaken for a remnant of the mesothoracic wing, though the vestige of wings, if there are any vestiges left in any Siphonaptera, should be sought for in the place where the wings of insects are inserted, namely, between the sternite and tergite, *i.e.* dorsally of the epimerum and not ventrally of it. The mesosternite has always some bristles on the side, there being usually one at the internal incassation, two at the stigma and one further dorsal, some species (for instance *regis* and *chephrenis*) having one or two more bristles.

The metanotum is sometimes shorter, and sometimes longer than the mesonotum in *Loemopsylla*, but the metasternite on the other hand is always larger than the mesosternite, the great development of the metathoracic epimerum being quite a special feature of the Siphonaptera. The metasternite has preserved the original sutures dividing it into three main sclerites, the fusion not having proceeded so far as in the case of the mesosternite. The meral suture is quite plain (see diagram, figs. A and B), and the anterior portion is again clearly divided by a horizontal suture into a sternum which is ventral, and an episternum which is lateral. The episternum is in *Loemopsylla* smaller than the sternum. It varies, however, a good deal, being, for instance, much longer in *L. longispinus* than in the other species of this genus. The most interesting feature in connection with this episternum of *Loemopsylla* is the more or less complete fusion with the sternum which obtains in a number of species, the suture between the two sclerites partially or totally disappearing both externally and internally in several species. In these species, therefore, the metasternite agrees more closely with the mesosternite than in the other species with a separate metathoracic episternum. This specialization we have found, outside the genera which are the subject of this paper, in an American *Ceratophyllus*<sup>1</sup> only. The fusion does not occur in the American non-combed *Pulicidae*, but is found in *Pulex irritans* and to a certain extent in the Old World species *Otenocephalus erinacei* and *Spilopsyllus cuniculi*. The third sclerite of the metasternite is the epimerum, which extends from the first abdominal tergite downwards to the hindcoxa, its

<sup>1</sup> *Ceratophyllus terinus* Rothsch (1905). In our revision of the *Sarcopsyllidae*, we stated (1906, p. 80) that the epimerum and sternum of the metathorax are fused as in *Ceratophyllus charlottensis*. This was a pen-slip for *C. terinus*.

lower edge covering part of the coxa. This episternum former authors considered to represent the hindwing of other insects, the real homology of this sclerite not occurring to them. Though its position is exactly as in the mesosternite the great size of the epimerum was apparently misleading. In all the genera of *Pulicidae* under discussion the epimerum has a great dorso-ventral extension, corresponding to the great expanse in that direction of the abdomen. The epimerum thus forms a lateral cover to the base of the abdomen replacing the absent sternal plate of the first abdominal segment. At the upper edge of the epimerum there is the third stigma. The epimerum bears in *Loemopsylla* always two rows of bristles<sup>1</sup>, the numbers varying often according to species, the females having a few more bristles than the males. The first row is sometimes represented by one bristle only (e.g. *creusae* and *isidis*). There is never an apical or subapical bristle on the epimerum<sup>2</sup>.

The abdomen (Pl. I) is strongly rounded dorsally and ventrally in both sexes of *Loemopsylla*, agreeing closely with that of *Pulex irritans*. The ventral curvature of the abdomen is much more marked in the male than in the female, especially anally, the genitalia being directed obliquely upwards. As in *Pulex* and *Ctenocephalus*, the abdomen is short, being more developed in a vertical direction than is the case in the American non-combed *Pulicidae*. The first tergite, which is the longest of all, but does not extend so far downwards as the other tergites, bears in most species of *Loemopsylla* two rows of bristles, the anterior row being sometimes represented by a few bristles only. In two species, however (*L. isidis* and *creusae*), this tergite has but one row of bristles. The tergites of segments 2 to 7 never bear more than one row of bristles, thus differing from the American non-combed *Pulicidae*, which have at least two rows on each segment. These bristles are placed on segments 2 to 6 of *Loemopsylla* a little beyond the centre of the segment, while on the seventh segment the row is situated much nearer the apical margin. In nearly all the species of *Loemopsylla* the row is complete, the most ventral bristle standing beneath the stigma, which is situated near the basal edge of the segment some distance from the row of bristles. The number of

<sup>1</sup> *L. aequisetorus* Enderl. is said to have only four bristles on this sclerite.

<sup>2</sup> On the inside of the epimerum or of the first abdominal tergite or of the meso- or metanotum there are often in mounted specimens the chitinous remnants of muscle-heads. We mention this because Packard (1894, p. 329) considered this patch to consist of hairs situated on the outside.

bristles is not the same in all the different species, the rows on the central segments containing, for instance, in *regis* about 20 bristles, while there are only about 10 in *pallidus*. The number is most reduced in *L. creusae*. In the male of this species the tergites bear 6 bristles on the two sides together, one placed below the stigma and the others on the back, the intermediate bristles being absent or reduced to minute hairs. The seventh tergite bears in addition to the postmedian series a single subapical bristle which is situated a short distance proximally of the apical edge of the segment (Pl. VI, figs. 1—4), as is the case also in *Pulex*, *Ctenocephalus*, *Moeopsylla* and *Parodontis*, the edge of the segment not being excised as in the other *Pulicidae* inclusive of the American forms allied to *Pulex* and *Loemopsylla* (Pl. VI, fig. 7). In the ♂ of *L. scopulifer* this bristle is placed on a process which projects beyond the edge of the segment (Pl. V, fig. 1).

The sternites of the third to sixth abdominal segments bear in *Loemopsylla* each a ventral row of bristles, there being sometimes a few additional bristles in front of this row on the second and third sternites or only on the third, as for example in *Loemopsylla erilli* and *gerbilli*. The first sternite, which is that of the second segment<sup>1</sup>, the first segment having no sternal sclerite in the Siphonaptera, as is also the case in many other insects, overlaps the second tergite, while in the other segments the upper portion is covered by the tergite. This first sternite has in most species of *Loemopsylla* only two ventral bristles, but in the ♀♀ of a few species (*L. longispinus*, *erilli* and *cheopis*) there are in addition some bristles on the side. The bristles on the sternite of the seventh segment are more numerous than on the preceding segments, there being on this segment a single row, and in some species a few additional hairs placed in front of the row.

<sup>1</sup> In *Journal of Hygiene*, VII. p. 446, there is an article on the morphology of *L. cheopis*, the name of the author of the essay not being stated. In this article the ninth sternite is correctly described, but the author, who apparently is not well acquainted with insect morphology, calls this plate the *seventh* sternite, treating the true seventh sternite as belonging to the sixth segment, the fifth sternite belonging to the fourth segment, and so on. He proceeds to explain this by maintaining that the basal sternite is that of the first segment and not of the second, it being quite erroneous to hold the opinion that the sternite of the first segment is absent. Some elementary knowledge of the composition of the abdomen of other insects and of the development of the segments in the chrysalis, we think, would doubtless have prevented the author from making such an astonishing statement. The opening of the vagina above the ninth sternite is quite the normal situation, the anus being situated between the tenth sternite and the tenth tergite. In the case of the ♂ the author makes a similar mistake. The "clasper" is called the ninth sternite, while it is really the lateral portion of the ninth tergite; and the tenth (or last) sternite has been overlooked altogether.

The eighth to tenth segments are modified, partly for sexual purposes. In the females, in which sex these segments are less complicated than in the males, the eighth tergite is very large, the sternite being reduced to a small elongate sclerite lying in between the ventral edges of the tergite (Pl. V, figs. 7—9). The tergite is much narrower dorsally than at the sides, where it is much dilated, and at the edge of the dorsal portion the large cavity of the stigma is situated. Above this stigma there are no bristles in *Loemopsylla* or only one or two very minute hairs, while the wide lateral portion of the segment bears numerous bristles. The number and position of these bristles are of taxonomic value, being more or less conspicuously different in the various species (Pl. V, figs. 7—9; Pl. VI, figs. 1—4). The sensory plate situated on the ninth tergite (Pl. VI, figs. 1—4) bears in *Loemopsylla* and allies on each side 14 setiferous grooves. There are fleas, however, in which the grooves are more numerous (for instance in *Chaetopsylla*). The dorsal outline of the plate is straight, and not much curved in lateral aspect. The ninth sternite is membranaceous laterally, extending far downwards, the most ventral portion being more strongly chitinized and forming a small sclerite. This plate lies inside the seventh or eighth segment and forms the ventral wall of the vaginal cavity, the duct of the *receptaculum seminis* ending above this plate (Pl. VII, fig. 4). The stylet of the anal (=tenth) segment is short and conical, being longer in *pallidus* than in the other species of *Loemopsylla*. The anal sternite is usually triangular in side-view (Pl. VI, figs. 1—4), bearing a long bristle ventrally before the apex and several shorter ones at the apex, there being also 3 or 4 bristles at the upper edge nearly equidistant from each other.

The sexually modified abdominal segments of the ♂♂ of the various members of the genus *Loemopsylla* (Pl. IV, figs. 6—12; Pl. V, figs. 1—6) exhibit a very uniform type of structure, differing in several details from those of all other genera, with the exception of *Parodontis* and *Moeopsylla*. The eighth tergite is small as compared with the eighth sternite, as is the case in all the allied genera. Its lower inner angle is not prolonged into a long slender manubrium as in *Pulex irritans*, being triangular in side-view. It slopes ventrally upwards, the ventral line being longer than the distance from the apex of the sternite to the upper corner of the stigma-cavity of the eighth tergite. The eighth sternite bears several bristles ventrally on each side, some species having only 2 or 3 bristles (e.g. *L. mycerini*, *erilli*, *ramesis* etc.), while others have a large number (e.g. *L. scopulifer*, *nubicus* etc.). The organs of copulation

project from the cavity formed by this sclerite. The genitalia are very small in *Loemopsylla*, the outline of the various projecting parts being difficult to make out in unmounted specimens except in a few species. These accessory copulatory organs are portions of the ninth segment. The central dorsal area of this tergite bears the sensory plate as in the ♀, the sides of the tergite being modified into clasping organs. This lateral portion, of which the main part may conveniently be called "clasper," is in *Loemopsylla* not separated from the dorsal portion by a suture. The lower inner angle of the clasper is produced inwards into a slender manubrium, which does not present any very striking differences in the various species of *Loemopsylla*, except in length (Pl. IV, fig. 6, M). Above the manubrium the inner edge of the clasper usually bears a slight tubercle-like projection. On the outer side of the clasper there are in *Loemopsylla*, *Moeopsylla* and *Parodontis* three processes ( $P^1$ ,  $P^2$ ,  $P^3$ ), of which two are movable, being connected with the clasper by a joint, while the third is often reduced or absent. These processes are very characteristic of the three genera mentioned, and are of great value in the discrimination of the species. They correspond to the large flap and the pair of pincers found in *Pulex irritans* (see Jordan and Rothschild, 1906, p. 38). The American non-combed eyed *Pulicidae* have a large clasper with one movable process, the manubrium being also large (Pl. VI, fig. 7). The ninth sternite is always strongly modified in Siphonaptera. In its most general form it is boomerang-shaped in side-view, consisting of an internal vertical, and a ventral horizontal arm, the latter projecting outside the eighth sternite. The vertical arm extends upwards to the base of the manubrium of the clasper, lying on the outer side of the manubrium, there being a vertical arm on each side of the abdomen. The horizontal arm is in *Pulicidae* either separated in the mesial line as far as the junction with the vertical arm, or there is no such separation into two ventral sclerites. In *Loemopsylla* as in *Pulex*, *Moeopsylla* and *Parodontis* the sternite is much reduced, the vertical arm sometimes not reaching the clasper. The ventral arm is divided into two slender processes, which are different in shape in the various species. This double process is moved by means of a long chitinous rod projecting far into the abdomen. The clasping organs appear to be always the same on both sides of the abdomen in Siphonaptera, apart from slight differences such as every two sclerites may present.

Between the clasper and the ninth sternite the penis projects, the chitinous parts of which are very complicated (Pl. IV, fig. 6, Pen.). The outline of the internal elongate plates—one on each side—varies more or

less according to species. The external portion of the organ also exhibits sometimes notable specific differences, for instance, the brush-like structure in *L. pallidus* being a very striking characteristic for that species (Pl. IV, fig. 9).

The differences found in the genitalia of both sexes of insects are of special importance to the systematist, since they enable him generally to recognise the species when other organs fail to exhibit sufficiently striking characteristics. In the study of Siphonaptera we lay great stress on the differences existing in these organs, conforming thereby to the general experience of entomologists in other groups of insects. The minute study of the genitalia of Siphonaptera is a great necessity, since these insects do not present so many and so varied external differences as is usually the case with the species of the orders of winged Arthropods.

As the senior author of the present essay has made special researches bearing on the question (1896, p. 426; 1905, p. 163), a short resumé of what is known of the constancy and variability of the external genitalia will perhaps be serviceable for those who are not acquainted with the taxonomic value of these organs. The discovery of the existence of differences in the genitalia of male insects is due to Léon Dufour (1844, p. 253). For a long time after entomologists held the opinion that the differences had been created or had developed for the purpose of preventing the species from intercrossing. These differences were considered constant within each species, and it was generally thought that specifically distinct insects exhibited in the genitalia some morphological distinction from their nearest allies. Inversely it was accepted as a fact that all forms of insects which had some characteristic in the genitalia were specifically distinct. These notions of the origin and significance of the genital differences in forms of insects were somewhat crude, the differences having since been reduced to their proper value. We know now (1) that the majority of species of insects show some morphological distinction in the genitalia from their congeners, there being no obvious difference of this kind in a small minority; (2) that the genitalia vary to a generally slight extent individually; (3) that the succeeding broods of an insect, though often very different in other organs, have the same genitalia, the only exception so far known being a butterfly (*Papilio aethus*) in the spring and summer broods of which the genitalia exhibit some slight and not quite constant differences between; (4) lastly, that the geographical forms of a species are very often different in respect to their genitalia (cf. Jordan, 1905, p. 151).

Now, the study of Siphonaptera is still in its infancy. We know next to nothing of the geographical variation of these insects; the study of that question has to be postponed till a sufficiently large amount of material from many countries is at disposal from which the geographical distribution of the various forms of Siphonaptera can be ascertained. That there is geographical variation also in Siphonaptera is proved to us by several species. It is, however, decidedly best to treat all forms which are constantly different as being specifically distinct, until more of the variation of the Siphonaptera is known.

The legs of *Loemopsylla* agree on the whole with those of *Pulex*, *Parodontis* and *Ctenocephalus*, differing essentially from the legs of the American non-combed eyed *Pulicidae* both in the internal rod of the mid- and hindcoxa dividing nearer the base of the coxa, and in the hindcoxa bearing a row of teeth on the inner surface. There is considerable variation in the details of the shape and in the amount of bristles of the legs within the genus *Loemopsylla*, a number of species being easily distinguished by the legs alone. The forking of the internal rod of the midcoxa near the base instead of in the centre of the coxa appears to us a character of considerable taxonomic value, being apparently a specialization obtaining only in some Old World genera.

As a rule the hind-edge of the mid- and hindcoxae of *Loemopsylla* is more or less excised before the apex, but there are also species in which this edge gradually slopes away. The coxae, for instance, in *cheopis*, *nubicus*, *pyramidis*, etc. have a distinct sinus, while in *isidis*, *creusae*, etc. the sinus is wanting and the hindcoxa of these species is pear-shaped (Pl. II, figs. 14—16). The sinus in *gerbilli*, *mycerini*, *erilli*, etc. is very shallow, the hind angle being completely rounded off. The number of spines in the comb of the hindcoxa is not the same in the various species of *Loemopsylla*, some species possessing only a few teeth, 4 or 5, while others have as many as 14 (*pyramidis*, for instance), but the number of teeth varies considerably within the species. The coxal comb is only found in some Old World genera of Siphonaptera and some Nearctic forms, but not in any Neotropical fleas. The femora present likewise some specific differences. The most notable distinction is that found in the hindfemur (Pl. II, figs. 6 and 7). This femur is flattened or grooved longitudinally on the ventral side, the edges of the flattened area converging anteriorly, and meeting at the point, where the ventral surface of the femur bears a distinct tooth-like projection. This obtains in six species (*pyramidis*, *cheopis*, *nubicus*, *cleopatrae*, *pallidus* and *eridos*). In the other species the femur is simply rounded, the tooth being also less



prominent in *cheopis* than in the other forms mentioned (Pl. I). The hindfemur in *Loemopsylla* always bears a row of hairs on the inner side. The tibiae are never hairy all over the outer surface. The relative length of the bristles of the tibiae and of the tarsal bristles, especially of those placed at the apex of the second hindtarsal segment, afford reliable distinctions, at all events between some of the species (Pl. III, figs. 7 and 8). The fifth tarsal segment bears 4 bristles on each side, besides a thin hair situated between the third and fourth bristles and placed a little more dorsad than these. Ventrally at the apex there are either 1, 2 or 3 short spine-like bristles, the number being sometimes not quite constant in the various individuals of a species (Pl. III, figs. 7, 8). The fore-, mid- and hindtarsi also differ as a rule in this respect.

24 species of *Loemopsylla* are known. The number will doubtless be greatly increased when the Siphonaptera of Asia and tropical Africa are better known. We are not acquainted with two of the species, described by Enderlein and Wagner respectively. They are placed in the present article where we think they belong according to the descriptions given by the authors, which are quite insufficient.

#### Key to the species of *Loemopsylla*.

- a. Frontal portion of head with 1 or 2 long bristles .....b  
     Frontal portion of head with 6 long bristles .....Species No. 24
  - b. Episternum and sternum of metathorax not separated from each other by a suture; hindfemur with or without tooth ventrally at the widest point .....c  
     Episternum and sternum of metathorax separated from each other by a suture; hindfemur with tooth ventrally at the widest point.....g  
     Episternum and sternum of metathorax separated from each other by a suture; hindfemur without a tooth ventrally at the widest point .....o
  - c. Hindfemur with a tooth ventrally at the widest point .....d  
     Hindfemur without this tooth .....Species No. 5
  - d. Fourth segment of hindtarsus short, triangular in outline .....e  
     Fourth segment of hindtarsus elongate .....f
  - e. Middle tergites of abdomen with a row of 8 or more bristles on the two sides together .....Species No. 1  
     Middle tergites of abdomen with a row of 6 or less bristles on the two sides together .....Species No. 2
  - f. Rostrum reaching to the trochanter .....Species No. 3  
     Rostrum not reaching to the trochanter .....Species No. 4
  - g. Subapical bristle of the seventh abdominal tergite in ♂ on a conical process which projects beyond the apex of the segment; seventh sternite of ♀ with about 15 bristles in front of the postmedian row on the two sides together ...Species No. 13  
     Subapical bristle of ♂ on a process, hindfemur in both sexes with 3 bristles on the outer side .....Species No. 14
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- Subapical bristle of ♂ not on a process ; seventh sternite of ♀ with many less than 15 bristles ; hindfemur with 2 bristles on the outer side .....h
- h. Subapical bristle of seventh abdominal tergite at least as long as the second hindtarsal segment.....i
- This bristle considerably shorter than the second hindtarsal segment .....n
- i. Epimerum of metathorax with 4 bristles.....Species No. 7
- Epimerum of metathorax with at least 10 bristles .....k
- k. Longest apical bristle of second hindtarsal segment hardly reaching the base of the fifth segment .....Species No. 10
- This bristle reaching to the middle of fifth segment or beyond .....l
- l. First midtarsal segment hardly two-thirds the length of the second.  
Species No. 9
- First midtarsal segment three-fourths the length of the second .....m
- m. Both processes of the clasper slender.....Species No. 8
- One of the two processes broad, its upper edge rounded.....Species No. 6
- n. On the epimerum of the metathorax at least one bristle situated above the stigma .....Species No. 12
- All the bristles of the epimerum of the metathorax ventral to the stigma.  
Species No. 11
- o. Hindcoxa pear-shaped, the comb situated near the apex .....p
- Hindcoxa more or less sinuate behind near the apex .....r
- p. Rostrum shorter than the maxillary palpus .....Species No. 15
- Rostrum longer than the maxillary palpus .....q
- q. Apical margin of the seventh abdominal tergite strongly chitinized dorsally, projecting backwards .....Species No. 17
- This portion of the segment hardly more chitinized than the rest of the segment, projecting very slightly backwards .....Species No. 16
- r. Rostrum reaching beyond the trochanter .....Species No. 18
- Rostrum at the most reaching to the apex of the forecoxa .....s
- s. Rostrum shorter than the forecoxa .....t
- Rostrum extending to the apex of the forecoxa .....Species No. 22
- t. First hindtarsal segment with 5 apical bristles which extend to the apex of the second segment or beyond .....Species No. 20
- First hindtarsal segment with less than 5 bristles which reach to the apex of the second segment .....u
- u. Rostrum longer than the maxillary palpus .....Species No. 19
- Rostrum as long as the maxillary palpus ; upper process of the clasper (♂) broad, truncate .....Species No. 21 (and 23 ♀)

### 1. Group of species.

Episternum and sternum of metathorax fused, the sternum either bearing a short hair or none (see diagram, p. 25). The fifth segment of all the tarsi has in both sexes two ventral apical bristles, one being long, the other short. Hindfemur angulate (with the exception of *L. longispinus*), bearing one subapical bristle on the outside. Manubrium of clasper short ; penis with a kind of brush at apex. To this group belong Species No. 1—5.

(1) *Loemopsylla pallidus* Taschenb. (1880).

(Pl. III, fig. 4; IV, fig. 9; V, fig. 8.)

*Pulex pallidus*, Taschenberg (1880 a, p. 65, n. 5, t. 1, fig. 9, Egypt, off *Herpestes ichneumon*); Baker (1895, p. 66, Socotra); Rothschild (1903, p. 542=*witherbyi*); Wagner (1903, p. 308); Tiraboschi (1904, p. 249); Baker (1905 a, p. 143, bibliography).

*Pulex witherbyi*, Witherby (1902, p. 60, indesscript).

*Pulex witherbyi*, Rothschild (1903, p. 86, n. 6, t. 1, figs. 2, 5, 6, t. 2, figs. 11, 15, White Nile and Shendi, off *Erinaceus albiventris*, etc.).

The insect recorded by Baker (1895, p. 66) from Socotra may possibly not be this species.

**Head.** The rostrum reaches beyond the apex of the forecoxa. The first segment of the maxillary palpus is a little longer than the third, and the second longer than the fourth. The most ventral bristle of the subapical row of the occiput is placed far apart from the next one, the second, or the second and the third, bristle being absent or replaced by a small hair. The first segment of the antenna of the ♂ bears 6 hairs along the hinder edge and an oblique row of about 7 across the segment ending at the anterior apical corner. In the ♀ the apical projection of the first antennal segment bears 2 hairs.

**Thorax.** The tergites bear 9 to 11 bristles on the two sides together. The pleura of the prosternite is pointed behind. The mesosternite bears 3 bristles. The internal rod of this plate, extending from the insertion of the coxa upwards, ends dorsally at the anterior corner of the sclerite, the portion of the plate situated in front of the rod being very narrow (side-view, see diagram, p. 25). The episternum of the metathorax is completely fused with the sternum, and of nearly the same size (lateral view), the line of separation being indicated posteriorly at the meral suture (which separates the epimerum from the sternum and episternum). There is one long bristle on the episternum, while the sternum bears only a small hair or no hair at all. The epimerum has two rows of bristles, the numbers being in ♂ 3 and 4, and in ♀ 4 or 5, and 4.

**Abdomen.** The first tergite bears an antemedian and a postmedian row of 4 (♂) or 5 (♀) bristles, there being also some minute hairs on the side between the two rows. The second to fourth tergites in ♀ usually bear 10 bristles on the two sides taken together, but sometimes only 8, in the ♂ the number being 9 or 10. On the fifth and sixth tergites there are 8 bristles in the ♀ and 8 or 9 in the ♂, while the seventh tergite has 6 bristles, two on each side being placed ventral to

the long subapical bristle. The most ventral bristle of the seventh tergite is far apart from the next. The sternites of segments 4 to 7 bear on both sides together usually 4 bristles, sometimes 5, the third segment having one or two more, while the basal sternite bears 1 to 3 ventral bristles.

**Legs.** The forecoxa has 16 to 18 bristles. The hindcoxa, which bears a comb of 8 to 12 spines, is rather strongly rounded behind, the sinus being very shallow. The forefemur has 4 to 6 bristles on the outer surface and 3 on the inner. The midfemur bears a row of 6 bristles on the inside, the hindfemur one of 7 to 15 (usually 8 in ♂ and 10 in ♀). All the femora have one subapical bristle on the outside. The tibiae have a single lateral row of bristles, the row containing 8 to 10 on the hindtibia. The hairs on the anterior surface (at and near the anterior edge in a lateral view) are very few in number. The longest apical bristle of the hindtibia reaches beyond the apex of the first tarsal segment. In the fore- and midtarsi (Pl. III, fig. 4) the first segment is shorter than the third; while the second midtarsal one is more than twice the length of the first. The bristles of the hindtarsi are long, the longest apical one of the first segment extending beyond the tip of the second, and that of the second segment reaching the apex of the fifth. The fifth segment is large in all tarsi, being much widened apically. The side-bristles of the segment are long, the interspace between the third and fourth bristles being wide, with a subdorsal hair in between them. The segment bears in all the tarsi two apical ventral bristles, both being slender, the one being short and the other long. The proportional lengths of the segments<sup>1</sup> are as follows:—

Segment	First	Second	Third	Fourth	Fifth
Foretarsus: ♂	8	12	9	5	22
	7	11	9	6	21
	9	15	11	7	25
	7	10	8	5	20
Midtarsus: ♂	12	24	18	8	24
	11	24	18	8	23
	16	32	16	9	26
	10	20	11	6	21
Hindtarsus: ♂	37	31	17	9	25
	34	30	16	9	25
	47	39	19	11	26
	30	26	18	8	22

<sup>1</sup> We give the measurements of a large and of a small specimen.

**Modified segments.** ♂. The eighth sternite (Pl. IV, fig. 9) bears 5 to 7 short bristles, 2 of them being placed near the apical margin. The clasper bears three processes, the central one being the narrowest, and the ventral one the shortest. The manubrium (M) is short, being about as long as (or even shorter than) the distance from its base to the tip of the longest process. The ninth sternite is narrow, and bent at the top into a short hook. The internal plate (endopodite) of the penis is broad, the apex being rounded off, with the upper corner remaining almost rectangular. The external part of the penis has the brush-like organ more strongly developed than that of any other species of *Loemopsylla*. — ♀. The apical edge of the eighth tergite (Pl. V, fig. 8) is nearly straight, bearing a row of bristles as shown in the figure, there being, besides, on the outer surface one long bristle on a level with the uppermost apical one and 2 or 3 on a level with the ventral apical bristles.

**Length.** ♂ 1·7—2 mm., ♀ 2·1—2·9 mm.

The junior author found this species rather commonly on various hosts in Egypt and the Egyptian Sudan. We have a long series from the following localities and hosts:

Egypt, off *Viverra ichneumon* (♂ and ♀, cotypes, received from the Berlin Museum).

Shendi, Egyptian Sudan, off *Erinaceus aethiopicus*, *Vulpes niloticus* and *Hyaena hyaena*.

Gebel Auli, White Nile, off *Erinaceus albiventris* (collected by H. F. Witherby).

Kerma, Dongola, off *Gerbillus pygargus* and *Vulpes vulpes aethiopicus*.

Cairo, off *Erinaceus auritus*.

Zaghig, Natron Valley, Lower Egypt, off *Erinaceus auritus* and *Canis zerda*.

Der Macarius, Natron Valley, Lower Egypt, off *Vulpes famelica*.

Karo Lola, Garre Livin country, South Somaliland, off *Erinaceus albiventris* (collected by Baron Carlo von Erlanger).

## (2) *Loemopsylla somalicus spec. nov.*

(Pl. III, fig. 8.)

This insect is closely allied to *L. pallidus*, differing, however, in the much smaller number of bristles.

**Head.** The subapical row of bristles of the occiput is represented on each side by a long ventral bristle and a small dorsal one, besides

some minute hairs. The first segment of the antenna bears in the ♂ 3 hairs at the hinder edge and close to the apex a transverse row of 4 or 5, the apical projection in the ♀ bearing one hair. There is a long bristle above the centre of the antennal groove.

**Thorax.** The epimerum of the metathorax bears very few bristles, the anterior row being represented by one bristle in the ♂, placed far downwards, and 2 in the ♀, while the posterior row contains 3 or 4 bristles. Each thoracic tergite bears 8 bristles on the two sides together.

**Abdomen.** The first tergite bears on the two sides together 4 postmedian bristles and before the centre a few minute hairs. There is no bristle beneath the stigma of the second to seventh tergites. The tergites 2 to 6 bear in the ♂ 4 bristles on the two sides together, the bristles being all dorsal, the more ventral ones usually situated in this position being replaced by minute hairs; in the ♀ the number of bristles on these segments is 6. On the seventh tergite there are 4 bristles in both sexes, one placed beneath the long subapical bristle.

**Legs.** The forecoxa has less than 15 bristles. The tibiae bear on the outer surface only one bristle, which is situated near the postmedian dorsal pair. The first and fourth midtarsal segments (Pl. III, fig. 8) are shorter than in *L. pallidus*. The fifth tarsal segment has no minute hairs on the ventral surface.

**Modified segments.** ♂. The first and third processes of the clasper are rather slenderer than in *L. pallidus*, the long subapical bristle being placed further away from the end. The ninth sternite is not so sharply hooked as in *L. pallidus*.—♀. The eighth sternite apparently as in *L. pallidus*.

We have both sexes from Karo Lola, Garre Livin country, South Somaliland, off *Sciurus spec.*, collected by the late Baron Carlo von Erlanger, on 5th May 1901.

### (3) *Loemopsylla cleopatrae* Rothsch. (1903).

(Pl. III, fig. 7; IV, fig. 7.)

*Pulex cleopatrae*, Rothschild (1903, p. 84, n. 3, t. 1, figs. 4, 8, t. 2, figs. 13, 17, Shendi, off various hosts); Baker (1905, p. 141); Balfour (1906, p. 104, fig. 58).

A small pale species, which is easily recognised by the long fourth hindtarsal segment.

**Head.** The rostrum reaches to the trochanter. The proportional lengths of the segments of the maxillary palpus are 9, 12, 8, 14. The

occiput bears a subapical row of 12 to 14 bristles on both sides together. The first segment of the antenna has in the ♂ a curved transverse row of about 9 hairs, the apical projection of this segment bearing in the ♀ one hair.

**Thorax.** The pronotum has 16 to 19 bristles on the two sides together, the mesonotum 16 or 17, and the metanotum 18 to 20. The mesosternite bears 3 to 5 bristles. There are 2 bristles and one or two minute hairs on the episternum of the metathorax; the sternum bearing a small hair. On the epimerum of the metathorax there are two rows of bristles, the anterior row containing 6 to 9, the posterior row 5 or 6, the most dorsal bristle of each row being placed above the stigma in both sexes.

**Abdomen.** The two rows of bristles on the first tergite contain each 8 to 10 bristles on the two sides together, the number of bristles on the other tergites being as follows:—ii. 19 to 21; iii. 19; iv. 20 or 21; v. 19; vi. ♂ 14 to 18, ♀ 20; vii. ♂ 14, ♀ 16. On the sternites of segments 3 to 7 there are in the ♂ 4 or 5 bristles, in the ♀ 6 to 8 (usually 8).

**Legs.** The forecoxa bears 22 to 26 bristles. The hindcoxa is broadly rounded, the sinus being rather deep and the angle behind it distinct. The femora have one subapical bristle on the outside, the midfemur bearing on the inside 2 bristles and the hindfemur 5. The first midtarsal segment is much longer than the third. In the hindtarsus the second segment is very slender and the fourth about two and a half times as long as it is broad (Pl. III, fig. 7). The longest apical bristle of the second hindtarsal segment nearly reaches to the apex of the fifth. The fifth segment is only slightly widened towards the apex. The proportional lengths of the segments are as follows:—

Segment	First	Second	Third	Fourth	Fifth
Foretarsus: ♂	7	8	5½	4	14
♀	9	10	6	5	15
Midtarsus: ♂	14	18	10	6	17
♀	20	25	14	7	18
Hindtarsus: ♂	40	36	20	18	19
♀	48	42	24	18	20

**Modified segments.** ♂. The eighth sternite has 5 or 6 bristles, of which three are situated along the ventral margin, the fourth is placed above the second ventral bristle, the fifth is much more dorsal, being situated at about two-thirds the distance from the ventral margin to the stigma, an occasional sixth bristle being situated between the fourth and

fifth. The clasper (Pl. IV, fig. 7) has two slender free processes, both bearing hairs at the apex, the upper one bearing also two longer bristles. A third, smaller, process, placed beneath the two free processes, is not separated from the body of the clasper. The manubrium is short and slender. The ninth sternite (ix. st.) is distinctly dilated at the apex, the ventral margin being there rounded. The internal plate of the penis is rounded at the apex.—♀. The apical edge of the eighth tergite is rounded. At the apical edge and above the ventral edge there is a row of 8 to 10 bristles, the apical margin bearing in addition from 5 to 8 smaller bristles which are placed on the inner side of the segment. On the lateral surface there are, besides, 5 to 10 bristles, of which two, rarely one, are placed beneath the stigma, but some distance from it.

**Length.** ♂ 1—1.4 mm., ♀ 1.4—1.7 mm.

This species was also found commonly by the junior author on various hosts in Egypt and the Sudan, as detailed below:—

Shendi, Sudan, off *Gerbillus pygargus*, *Dipodillus watersi*, *Jaculus jaculus*, *Lepus aethiopicus* and *Erinaceus aethiopicus*.

Kerma, Dongola, off *Gerbillus pygargus*.

Shereik, N. Sudan, off *Gerbillus pygargus* and *Acomys witherbyi*.

Khartoum, off *Gerbillus gerbillus* (collected by Dr A. Balfour).

Albumar, Bir Victoria, Natron Valley, off *Ictonyx libyca*.

Zaghig, Natron Valley, off *Gerbillus tarabuli* and *Jaculus jaculus*.

Bir Victoria, Natron Valley, off *Lepus rothschildi* and *Gerbillus tarabuli*.

Mt Mulluk, Natron Valley, off *Meriones sellysii*.

#### (4) *Loemopsylla pyramidis* Rothsch. (1904).

*Pulex pyramidis* Rothschild (1904 b, p. 3, n. 3, Natron Valley, Lower Egypt, off *Jaculus jaculus*); Baker (1905, p. 143).

Only one ♀ is known to us. The specimen very closely resembles that sex of *L. cleopatrae* and is perhaps only an exceptionally large individual of that species. The rostrum, however, does not reach the trochanter; the episternum of the metathorax bears only one long bristle, besides some minute hairs; and the hindtibia has two additional lateral bristles between the lateral row and the dorsal edge. The ♂ might possibly be abundantly distinct from *L. cleopatrae*.

One ♀ off *Jaculus jaculus*, found by the junior author at Bir Victoria, Natron valley, Lower Egypt, on March 9th 1903.



(5) *Loemopsylla longispinus* Wagn. (1893).

(Pl. III, fig. 9.)

*Pulex longispinus* Wagner (1893, p. 355, t. 6, fig. 1, ♀, West Turkestan, off *Erinaceus spec.*); Baker (1904, p. 437).

This is the only species known in which the episternum and sternum of the metathorax are fused, while at the same time the hindfemur is non-angulate ventrally near the base.

**Head.** The rostrum does not quite reach the trochanter. The first segment of the maxillary palpus is longer than the third, while the second is longer than the fourth. There is a wide interspace between the first and second bristles of the subapical row on the occiput. The apical projection of the first antennal segment of the ♀ is broad, bearing one hair at the tip.

**Thorax.** The bristles on the three tergites, on the two sides together, are respectively 17, 20, and 17. The mesosternite bears 3 bristles. The episternum of the metathorax has one bristle, while the epimerum bears two rows of 5 each, the bristles of the posterior row being very long.

**Abdomen.** The anterior row of bristles on the first tergite consists of 4 and the posterior row of 8 bristles on the two sides together, the number of bristles on the following segments being 15, 14, 12, 12, 10 and 10. The basal sternite bears a ventral pair and about 5 more bristles on each side, the other sternites having a single row consisting of 11, 8, 6, 6 and 6 bristles respectively.

**Legs.** The comb of the hindcoxa contains 14 or 15 spines. The bristles on the upper side of the femora are exceptionally long. The mid- and hindfemora have one subapical bristle on the outer side, bearing a row of 8 and 9 respectively on the inner side. The bristles of the tibiae are very long and strong (Pl. III, fig. 9). The longest apical bristle of the hindtibia extends beyond the apex of the first tarsal segment. In the foretarsus the second segment is twice as long as the first, while the first segment of the midtarsus is more than twice the length of the first. There are 6 bristles at the apex of the first hindtarsal segment which reach to the apex of the second or beyond, while there are 4 apical bristles on the second segment which reach beyond the middle of the fifth. The fifth segment is dilated at the apex, the lateral bristles being long and stout.

**Modified segments.** ♀. The apical margin of the eighth tergite is nearly straight. There is a row of 9 bristles along the apical and

ventral margin, 2 more bristles being placed on the lateral surface. On the inside of the apical margin there are 16 bristles and hairs.

The above description is taken from a ♀ received from Dr J. N. Wagner, of Kieff. The ♂ is not yet known.

The insect has so far only been found on *Erinaceus auritus* in West Turkestan.

## 2. Group of species.

Episternum of metathorax separated from sternum (see diagram, p. 25), the latter bearing a bristle which is as long as that situated on the episternum. Hind-femur angulate ventrally at the widest point, fifth segment of fore- and midtarsi with three spine-like bristles ventrally at apex, the lateral ones being very stout in ♂. Clasper with two distinct free processes; manubrium long; penis without a kind of brush near apex. To this group belong Species No. 6—13.

### (6) *Loemopsylla cheopis* Rothsch. (1903).

(Pl. I; II, fig. 8; IV, fig. 8; VI, fig. 1.)

*Pulex cheopis* Rothschild (1903, p. 85, n. 4, t. 1, figs. 3, 9, t. 2, figs. 12, 19, Shendi, off *Acomys*, *Arvicanthia*, etc.); Wagner (1903, p. 308); Baker (1905 a, p. 141); Advisory Committee (1906, p. 421, history of plague investigation, literature); Rothschild (1906 b, p. 483); Advisory Committee (1906, p. 486, t. 9, mouth-parts and alimentary canal); Advisory Committee (1907, pp. 446, 472, t. 10—12, morphology); Tiraboschi (1907, pp. 570, 581).

*Pulex brasiliensis* Baker (1904, p. 379, São-Paulo, off *Mus rattus* and *decumanus*); Baker (1905 a, p. 129).

*Pulex murinus* Tiraboschi (1904, p. 251, fig. 15, ♂, Italy, off rats); Tiraboschi (1907, p. 570, = *cheopis*).

*Pulex philippinensis* Herzog (1904, p. 77, figs. 26, 27, Manila, off rats); Tiraboschi (1907, p. 582, "not proved to be the same as *cheopis*").

**Head.** The rostrum reaches to the apex of the forecoxa. The proportional lengths of the segments of the maxillary palpus are 12, 15, 11, 20. The subapical row of bristles of the occiput contains 6 bristles on each side. The first antennal segment of ♂ bears 4 hairs at the hinder edge and a transverse row of about 7, there being proximally to this row 2 or 3 additional hairs. In the ♀ the apical projection of the first antennal segment has 2 or 3 hairs at the tip, and about 4 minute ones on the outer surface of the segment as well.

**Thorax.** The number of bristles in the row on the pronotum varies from 13 to 16, on the mesonotum from 12 to 14, and on the metanotum from 11 to 12, the bristles of the two sides being counted together. The mesosternite bears 5 bristles on the side, the episternum of the metathorax one, and the epimerum 10 to 17 in two rows (5 to 8, 5 to 7).

The ventral angle of the metasternum is acuminate (see diagram, p. 25).

**Abdomen.** The first row of bristles on the first tergite consists in the ♂ of 5 to 7 bristles, in the ♀ of 6 to 10, the usual number being 6 in both sexes. The second row contains in the ♂ 6, in the ♀ 6 to 10 (usually 6) bristles. The numbers of bristles on the other tergites are as follows:—ii. ♂ 13 to 15, ♀ 15 to 21; iii. ♂ 14 to 15, ♀ 15 to 23 (usually 15 to 17); iv. ♂ 14, ♀ 14 to 23 (usually 15 or 16); v. ♂ 13 or 14, ♀ 15 to 22 (usually 15 or 16); vi. ♂ 15 or 16, ♀ 13 to 20; vii. ♂ 12 or 13, ♀ 12 to 17 (usually 14 or 15). The dorsal subapical bristle of the seventh segment is much longer than the second hindtarsal segment. The bristles on the sternites are as follows:—ii. 2 in ♂ and ♀; iii. ♂ 6 or 7, ♀ 9 to 12 (usually 10); iv. ♂ 6 to 8, ♀ 8 to 13; v. ♂ 6, ♀ 8 to 12; vi. ♂ 6 to 8, ♀ 8 to 12; vii. ♂ 6, ♀ 10 to 16, on this segment there being also one or more small hairs in front of the row.

**Legs.** The forecoxa bears about 30 or 32 bristles. The apical sinus of the hindcoxa is rounded, the angle behind it being distinct. The comb of the hindcoxa consists of 5 to 9 spines. The forefemur has 4 to 8 hairs on the outer surface, besides one subapical bristle. On the hindfemur there is on the inside a row of 5 to 9 bristles and on the outside 2 subapical bristles. The hindtibia bears on the outer surface 7 or 8, rarely 9, bristles, and two or three additional bristles between this row and the dorsal edge. The longest apical bristle of the hindtibia reaches to the subapical notch of the first tarsal segment. The second midtarsal segment is twice as long as the third. In the hindtarsus the fourth segment is twice as long again as it is broad. The longest apical bristle of the second segment extends to the middle of the fifth. The fifth segment is only slightly dilated towards the apex, bearing in the fore- and midtarsi three apical bristles on the ventral surface, the two lateral ones being short and stout in the ♂. In the hindtarsus this segment bears in both sexes two ventral apical bristles, one being short and the other long. The second hindtarsal segment is proportionately longer in the ♂ than in the ♀. The proportionate lengths of the segments are as follows:

Segment	First	Second	Third	Fourth	Fifth
Foretarsus: ♂	9	10	8	6	17
♀	11	12	9½	7	20
Midtarsus: ♂	16	23	18	7	20
♀	23	26	13	9	21
Hindtarsus: ♂	47	32	18	11	22
♀	53	36	19	12	24

**Modified segments.** ♂. The eighth sternite (Pl. IV, fig. 8) bears 4 or 5 long bristles along the ventral edge, the last two being the longest; besides these there are a number of bristles on the side as shown in the figure. The manubrium (M) of the clasper is longer than the distance from its base to the top of the longer process of the clasper. There are two free processes to the clasper, the upper one being somewhat broad and asymmetrical, its upper margin being rounded, and the lower margin somewhat incurved, the bristles situated at and near the apex and upper margin being long. This process is very distinct from that of every other species known to us. The ninth sternite (ix. st.) almost gradually widens from the base to the apex, the upper margin being nearly straight and the ventral margin bearing a number of small hairs from near the base to the apex. The internal plate of the penis is somewhat curved upwards at the end, the ventral margin being rounded and the upper corner pointed.—♀. The eighth tergite (Pl. VI, fig. 1) bears externally along the apical edge a row of 12 to 16 (usually 13) bristles, and a row of 8 or 9, rarely 10, shorter ones inside, on the outer surface there being an irregular row of usually 8 to 10 bristles which are placed between the lateral and apical rows. The stylet is short, bearing, besides the long apical bristle, one or two short ones situated on the outer surface.

**Length.** ♂ 1.4–1.7 mm., ♀ 2.1–2.7 mm.

This species appears to occur in all warm climates, being distributed by rats. It is presumably the means of the transmission of the bubonic plague from rat to man. The true home of this flea appears to be the Nile Valley, where it occurs commonly on various hosts.

Though we have not seen the specimens which were named *brasilensis* and *philippinensis* (see bibliography, above), we have little doubt that the names refer to the present insect.

*L. cheopis* formerly was generally referred to as *Pulex pallidus* Tasch. in the literature on bubonic plague.

In most of the ♀♀ off *Gerbillus* and *Jaculus* as well as in the single one we have off *Lepus rothschildi*, the hindcoxa is broader than is the case in typical *cheopis*. The first midtarsal segment, moreover, is longer and the tooth of the hindfemur larger. These specimens are possibly the ♀♀ of the two ♂♂ mentioned under *L. nubicus*.

We have *L. cheopis* from the following localities and hosts:

Shendi, Egyptian Sudan, off *Acomys witherbyi*, *Arvicanthis testicularis*, *Dipodillus watersi* and *Genetta dongolana*.

Meroe, Dongola, off *Arvicanthis testicularis*.

Pretoria, Transvaal, off *Mus decumanus*.  
 Beira, East Africa, off *Mus chrysophilus*.  
 Entebbe, Uganda, off man.  
 Benguela, Angola, off *Mus rattus* and *Funisciurus spec.*  
 Plaines des Palmes, Réunion, off *Crocidura murina*.  
 Marseille, France, off rats.  
 Plymouth, England, off a rat.  
 Adana, Asia Minor, off *Mus decumanus* and *Mus spec.*  
 Muscat, Arabia, off *Mus decumanus*.  
 Bombay, off deer.  
 Agra, India, off rats.  
 Jacobabad, India, off *Mus rattus rufescens*.  
 Loo Choo Is., Japan, off *Crocidura caerulea*<sup>1</sup>.  
 Freemantle, West Australia, off *Mus decumanus*.  
 Paramatta, New South Wales, off *Mus decumanus*.  
 Onaca, Santa Marta, Colombia, off rats.  
 Sapucay, Paraguay, off *Mus rattus* and *alexandrinus*.  
 San Bernardino, Paraguay, in a cellar frequented by rats.

(7) *Loemopsylla aequisetosus* Enderl. (1901).

*Pulex aequisetosus* Enderlein (1901, p. 554, fig. B, t. 35, fig. 7, ♀ 10, Togoland, off *Cricetomys*); Wagner (1903, p. 309, "descript. insufficient"); Baker (1905 a, p. 140).

We have not had an opportunity of examining the specimen upon which the name is based. This specimen being unique, the authorities of the Berlin Museum were not allowed to lend it to us. The director of that institution, however, has kindly supplied us with information of the more important details respecting the structure of this insect, which are not mentioned in the original description nor shown in the figures accompanying the same.

According to this additional information the episternum of the metathorax is separated from the sternum, and the hindfemur bears a minute ventral tooth. This insect, therefore, belongs to the present group of species. In the original description the segments of the maxillary palpus are said to be almost the same in length, while we are now informed that the proportional lengths are 10, 13, 9, 14. The epimerum of the metathorax bears 4 bristles, according to our informant. The tergites of the thorax and abdomen bear each a row of

<sup>1</sup> These large shrews like rats inhabit ships and consequently transported from place to place.

about 10 to 12 bristles according to Enderlein, there being an additional row on the first abdominal tergite. The sternites of the third to seventh abdominal segments have each a row of about 6 to 8 bristles. The rostrum is longer than the maxillary palpus.

The insect is apparently closely allied to *L. cheopis*.

Mangu, Togoland, West Africa, August 1898, off a species of *Cricetomys spec.*; one ♀ in the Berlin Museum.

(8) *Loemopsylla nubicus* Rothsch. (1903).

(Pl. III, fig. 6; IV, fig. 6.)

*Pulex nubicus* Rothschild (1903, p. 84, n. 2, t. 2, figs. 10, 16, Shendi, off various hosts); Wagner (1903, p. 308); Baker (1905 a, p. 142).

Closely resembling *L. cheopis*. We are not sure that we have the ♀. The specimen which we originally described as the ♀ of *nubicus* now appears to us to belong to *cheopis*. There are no specimens among our material which with safety can be regarded as the ♀♀ of *L. nubicus*.

The ♂ is easily distinguished from *L. cheopis* by the genitalia. The clasper (Pl. IV, fig. 6) bears two slender processes, the shorter process having 3 to 5 bristles at the apex, of which the one occupying the tip is the longest. On the dorsal side there is another pair of hairs, placed at two-thirds or three-fourths the distance from the base. The horizontal portion of the ninth sternite (ix. st.) is transparent, being very slightly chitinized, except its ventral margin and base. Along the ventral margin there are from 10 to 13 hairs. The internal plate of the penis (Pen.-Pl.) is broad, its apex being rounded, with the upper corner slightly angulate.

Length. ♂ 1.4—2 mm.

Our series of ♂♂ collected by the junior author are as follows:

Shendi, Egyptian Sudan, February and March 1901, off *Arvicanthis testicularis*, *Herpestes albicauda*, *Genetta dongolana*, and *Gerbillus robustus*.

Nakheila, Atbara R., no host given.

Bir Victoria and Zaghigh, Natron Valley, Lower Egypt, March 1903, off *Jaculus jaculus*.

We have also two ♂♂ off *Gerbillus gerbillus* and *Jaculus jaculus*, which are rather larger than the ones mentioned above; their hindcoxa is broader, the first midtarsal segment a little longer and the tooth of the hindfemur rather more strongly developed. The genitalia are

apparently not different from those of typical *nubicus*. The aberrant ♀♀ mentioned under *cheopis*, possibly belong to these ♂♂. The material is not extensive enough to enable us to decide whether these specimens are merely large individuals of *nubicus*, or whether they represent a distinct species. The relatively broad hindcoxa they share with *chersinus*. In the latter insect, however, the first midtarsal segment is much shorter.

(9) *Loemopsylla chersinus* Rothsch. (1906).

(Pl. III, fig. 5; V, fig. 5.)

*Pulex chersinus* Rothschild (1906 a, p. 75, t. 4, fig. 1—3, Khartoum, off *Jaculus gordonii*).

Closely allied to *L. nubicus* and *cheopis*. The subapical bristle of the seventh abdominal tergite is as long as the second hindtarsal segment. The hindcoxa is broader than in the allied species, bearing a comb of five spines. The first midtarsal segment is short, being only two-thirds the length of the second (Pl. III, fig. 5). The genitalia resemble those of *nubicus*; but the most proximal bristle of the broader process is situated much nearer the apex (Pl. V, fig. 5).

We have only one ♂ (the type) found on *Jaculus gordonii* at Khartoum by Dr A. Balfour.

(10) *Loemopsylla nesiotus* spec. nov.

(Pl. III, fig. 3; VI, fig. 4.)

Larger than *L. nubicus*; the bristles of the tibiae and tarsi much stouter and shorter.

**Head.** The rostrum reaches to the trochanter. The first segment of the maxillary palpus equals the third, the proportions of the four segments being 14 to 16, 18 to 19, 14 to 16, and 24.

The first antennal segment of the ♂ bears 4 minute hairs at the hind edge, a transverse row of 7 or 8, and proximally to this row 1 or 2 additional hairs. Above the antennal groove there are, in ♂, 15 or 16 small hairs.

**Thorax.** The pronotum bears, on the two sides together, 13 to 15 bristles, the mesonotum 13 or 14, and the metanotum 12 to 14. There are usually 5 bristles on the pleura of the mesosternite, but occasionally only 4. The episternum of the metathorax bears 12 to 14 bristles arranged in two rows (6 to 9, 5 to 6).

**Abdomen.** The first row on the first tergite contains 6 or 7 bristles, and the second row 6 on both sides together, the numbers of bristles on the other tergites being on the two sides together, 16 or 17, 16, 15 or 16, 14, and 13 or 14. On the sternites of segments 2 to 7 the numbers of the bristles are in the ♂ 2, 7 or 8, 7 to 9, 10 or 11, 8 to 10, and 10, there being 2 to 4 small hairs in front of the row on the seventh segment. In the ♀ the numbers of bristles on the sternites 3 to 7 are rather larger than in the ♂. The dorsal subapical bristle of the seventh sternite is nearly as long as the first and second midtarsal segments together.

**Legs.** On the forecoxa there are close on 40 bristles. The forefemur bears on the outer side 6 to 8 hairs. On the midfemur there are laterally 4 or 5 subventral bristles on the inside and 2 subapical ones on the outside, while the hindfemur has 5 to 7 on the inside and 2 on the outside. The bristles of the tibiae and tarsi are stout, being shorter and much stronger than in *L. nubicus* and also much shorter than in *L. cheopis* and *eridos*. The longest apical bristle of the hindtibia does not reach the subapical bristles of the first hindtarsal segment (Pl. III, fig. 3). The longest apical bristle of the first hindtarsal segment does not extend to the apex of the second segment, while the longest dorsal apical bristle of the second segment only reaches to the base of the fourth segment, the longest bristle on the anterior side of the second segment not even extending to the apex of the third segment, these bristles, therefore, being much shorter than in the allied species. The fifth segment bears 3 ventral apical spines in the fore- and midtarsi, and a long and short one instead in the hindtarsus, these bristles being stout in the ♂ and slender in the ♀.

#### CORRECTION

p. 48, second line from the bottom,

*instead of "C. W. Andrews" read "Dr H. E. Durham."*



(11) *Loemopsylla eridos* Rothschild. (1904).

(Pl. VII, fig. 4.)

*Pulex eridos* Rothschild (1904, p. 611, n. 6, t. 8, fig. 21, t. 9, fig. 23, Deelfontein, off *Otomys brantsi*); Baker (1905 a, p. 141).

**Head.** The rostrum does not quite reach the trochanter. The first segment of the maxillary palpus is longer than the third, the second being a little shorter than the fourth. The subapical row of bristles of the occiput bears 5 or 6 bristles on each side. The first antennal segment of the ♂ is less produced posteriorly at the apex than in *L. cheopis* and *nubicus*; it bears 3 hairs at the hinder edge, and a transverse row of 5, proximally to which there are 2 more hairs. In the ♀ the projection of the first antennal segment bears one hair.

**Thorax.** The nota of the thorax bear usually 12 bristles on both sides together, the pronotum having sometimes 14 and the metanotum occasionally 11. The sternite of the mesothorax bears 3 hairs. The episternum of the metathorax has one bristle, while the epimerum bears an anterior row of 4 to 6 and a posterior row of 6.

**Abdomen.** The irregular anterior row of the first tergite on both sides together contains 7 to 10 bristles and the posterior row 6. The second to fifth tergites bear in the ♀ 16 to 18 bristles on both sides taken together, the sixth 15 or 16, and the seventh 12, the ♂ having 1 to 3 bristles less on the second to fifth tergites. The numbers of bristles on the sternites are as follows:—ii. ♂ 2, ♀ 2; iii. ♂ 4, ♀ 8; iv. ♂ 5 or 6, ♀ 8; v. to vi. ♂ 5 or 6, ♀ 8; vii. ♂ 7, ♀ 6 to 8, the seventh segment bearing in ♀ some additional short bristles in front of the row. The dorsal subapical bristle of the seventh tergite is one-

first hindtarsal segment does not extend to the apex of the second segment, while that of the second segment reaches beyond the middle of the fifth. The fourth hindtarsal segment is about half as broad again as it is long. The fifth segment is small, being very slightly dilated towards the apex. In the hindtarsus this segment is shorter than the second. There are in both sexes on the ventral side of the fifth segment 3 apical bristles on the fore- and midtarsi and 2 in the hindtarsi, these bristles being thinner than in *L. cheopis*.

**Modified segments.** ♂. The eighth sternite bears about 10 bristles on each side. The clasper has two processes; the shorter one is cylindrical, bearing long bristles from near the base to the apex. The ninth sternite is obtuse at the tip, bearing here a number of fine hairs, there being also several at the ventral margin. The internal plate of the penis is curved upwards at the apex, which is pointed.—♀. The apical margin of the eighth tergite is rounded; the segment bears on the outside an apical row of about 10 bristles, besides about 10 lateral ones. The ninth sternite, which lies inside the seventh, is different from that of the other species of *Loemopsylla*. Above the ventral edge of this sternite (Pl. VII, fig. 4, r. st.) there is a bracket-like rod, which is the chitinized apical portion of the duct of the receptaculum seminis. At the tip of this rod there are two short, but strongly chitinized sclerites which most likely serve as a clasping organ during copulation.

We have a series of specimens collected by C. H. B. Grant, as follows: Deelfontein, Cape Colony, March 1902, off *Otomys brantsi*; Umfolozi, Zululand, July 1904, off *Mus spec.*

(12) *Loemopsylla niloticus spec. nov.*

(Pl. V, fig. 3.)

Closely allied to both *L. nubicus* and *eridos*, but nearest to the latter, differing especially in the bristles of the episternum and epimerum of the metathorax and the first abdominal sternite.

**Head.** The apical corner of the first antennal segment of the ♂ is less produced than in *L. nubicus* and *cheopis*, the hairs on this segment, however, are placed nearly as in *cheopis*.

**Thorax.** The pronotum bears 17 to 19 bristles on the two sides together, the mesonotum 14 to 16, and the metanotum 16, the numbers varying to a certain extent, as in other species. There are 5 bristles on the side of the mesosternite. The episternum of the metathorax

bears 2 bristles, there being only one in the allied species. On the epimerum of the metathorax there are two rows of bristles, the first containing from 7 to 10 and the second from 6 to 8: the first bristle of the anterior row, or of both rows, being dorsal to the stigma, which is not the case in the other species of the present group of *Loemopsylla*.

**Abdomen.** The numbers of bristles on the tergites are as follows (on both sides together):—i. (two rows) 9 to 11 and 9; ii. 22 to 28; iii. 23 to 28; iv. 23 to 28; v. 22 to 26; vi. 20 to 27; vii. 17 to 22, the ♂ usually having a few bristles less than the ♀. The basal sternite bears in both sexes two ventral pairs of bristles, the one pair standing in front of the other; in the ♀ there are, moreover, 2 lateral bristles on each side, these bristles being replaced in the ♂ by minute hairs. None of the allied species have more than one ventral pair of bristles. On the following sternites the bristles are as follows:—iii. ♂ 10, ♀ 14 or 15; iv. ♂ 10, ♀ 14; v. ♂ 10, ♀ 16; vi. ♂ 10, ♀ 15 or 16; vii. ♂ 10, ♀ 15 or 16, there being in front of the row of the seventh segment a few minute hairs in ♂ and 7 to 12 bristles in the ♀ on both sides together. The dorsal subapical bristle of the seventh tergite is shorter than the second hindtarsal segment.

**Legs.** The hindfemur bears a row of 5 or 6 bristles on the inside, and has 2 subapical bristles on the outside.

**Modified segments.** ♂. The bristles on the eighth sternite are rather more numerous than in *L. cheopis*. The clasper bears two slender processes (Pl. V, fig. 3). The upper (= outer) process has 3 or 4 bristles at the tip and 3 more between the apex and the centre along the upper margin. The ninth sternite resembles that of *L. cheopis*, bearing a number of hairs at the apex and a very few additional ones further proximad and near the ventral margin. The internal plate of the penis (Pen.-Pl.) is slender, its apex being curved upwards and acuminate.—♀. The bristles on the eighth tergite are very numerous, the long bristles situated on the lateral surface being accompanied in the present species by an unusually large number of shorter bristles.

The junior author found a number of specimens of both sexes in Egypt and the Sudan on various hosts, as detailed below:

Nakheila, Atbara R., February 1904, off *Gerbillus robustus*.

Shereik, N. Sudan, January 1904, off *Gerbillus pygargus*.

Shendi, Sudan, March 1901, off *Arvicanthis testicularis* and *Gerbillus tatera*.

Kerma, Dongola, February 1904, off *Gerbillus pygargus*.

(13) *Loemopsylla scopulifer* Rothschild. (1905).

(Pl. V, figs. 1 and 9.)

*Pulex scopulifer* Rothschild (1905, p. 480, n. 2, t. 13, fig. 5, Umfolozi, South Africa, off *Saccostomus campestris*).

The ♂ differs abundantly from all the other species of *Loemopsylla* in the structure of the genitalia, as well as in the long subapical bristle of the seventh abdominal tergite being placed on a conical process, while the ♀ is distinguished by the stout bristles of the legs and the large number of bristles situated on the seventh abdominal sternite.

**Head.** The subapical row of the occiput contains 6 or 7 bristles on each side. There are 2 hairs at the tip of the conical projection of the first antennal segment of the ♀.

**Thorax.** The pro-, meso-, and metanota on the two sides together bear in the ♀ 16 bristles, while there are one or two less in the ♂. There are 5 or 6 bristles on the pleura of the mesothorax. The episternum of the metathorax has one bristle, and the epimerum in the ♂ usually 14 (7, 7) and in the ♀ 14 to 16 (9, 7 or 8, 6).

**Abdomen.** The number of bristles on the tergites are as follows (on the two sides together):—i. 14 to 16 in two rows (= 7, 7 or 8, 8); ii. 18 or 19; iii. 18 to 21; iv. 16 to 20; v. 17; vi. 16; vii. 13 to 15. The dorsal subapical bristle of the seventh tergite which in the ♂ stands on a prominent conical process (Pl. V, fig. 1) equals in length the first and second midtarsal segments. On the sternites there are the following bristles:—ii. 2; iii. ♂ 9, ♀ 12; iv. ♂ 10, ♀ 12; v. ♂ 10, ♀ 10; vi. ♂ 10, ♀ 10 to 15; vii. ♂ 14, ♀ 18. On the seventh segment there are, moreover, in the ♂ about 3 hairs in front of this row, and in the ♀ about 15 or even more, on the two sides together.

**Legs.** The forecoxa bears about 37 bristles. The midfemur has 3 bristles on the inside and the hindfemur 4 or 5, while both have 2 subapical bristles on the outside. The bristles of the tibiae are stout. The shortest dorsal apical bristle of the foretibia is very blunt and thick, the other apical bristles being also less pointed than usual. The longer curved apical bristle of the hindfemur is shorter than the hindtibia is broad at the apex. In the ♂ the longest apical bristle of the hindtibia extends to the subapical notch of the first tarsal segment, while it reaches in the ♀ to the centre of the segment. The longest apical bristle of the second hindtarsal segment extends to the apex of

the fourth or base of the fifth segment, the longest bristle of the anterior side reaching only to the apex of the third segment. The fifth segment in the fore- and midtarsi of the ♂ has 3 ventral apical spine-like bristles, the central one being the longest, the lateral ones being short and very stout. In the ♀ this segment has, in contradistinction to the ♂, one long and one short bristle.

**Modified segments.** ♂. The eighth sternite bears about 22 bristles on each side. The clasper (Pl. V, fig. 1) has a short and a long process, the short one bearing some very stout bristles. The ninth sternite is stouter than is usual in this genus, the apex being hairy. The internal plate of the penis is pointed, the external plate becoming more and more acuminate towards the apex.—♀. The eighth tergite (Pl. V, fig. 9) has an external apical row of 10 to 12 bristles and an internal apical row of 10 short ones; there is, on the external surface also, a row of 8 to 10 bristles extending from the stigma downwards, 2 long bristles standing in between the lateral and apical rows.

**Length.** ♂ 1.5—2 mm., ♀ 1.7—2 mm.

A South African species, of which we have a series of both sexes as follows: Umfolozi Station, Zululand, taken in July and September 1904, off *Saccostomus campestris*, and also off *Mus auricomis*, September 1904; Beira, Portuguese East Africa, February 1907, off *Cricetomys gambianus*; all collected by C. H. B. Grant.

(14) *Loemopsylla tortus* spec. nov.

(Pl. V, fig. 4.)

Very closely allied to the preceding species. Both sexes, however, are larger. The hindfemur bears 3 subventral bristles on the outer side before the apex. The bristles situated at the apex of the hindtibia are longer than in *L. scopulifer*, the longest bristle reaching in the ♂ to the apex of the first hindtarsal segment and in the ♀ to the subapical notch of the same. The seventh abdominal sternite of the ♀ bears only 6 to 8 bristles in front of the postmedian row of bristles. The larger process of the clasper of the ♂ (Pl. V, fig. 4) has 6 bristles as in *L. scopulifer*, but these bristles are shorter and much thinner than in that species. The second process of the clasper is narrowed towards the apex and gradually curved upwards. The largest bristle of the anal tergite of the ♂ is longer than in *scopulifer*, as are also the bristles situated on the eighth sternite.

We have a small series of both sexes from Cajuno, Inhambane district, S.E. Africa, off *Mus spec.*, August 1906, and from Beira, S.E. Africa, off *Cricetomys gambianus*, February 1907, collected by C. H. B. Grant.

In the tube containing the specimens taken off *Cricetomys gambianus* there are both this species and *L. scopulifer*. The two insects, therefore, appear to exist side by side and must be regarded as distinct species.

### 3. Group of species.

Episternum of metathorax separated from the sternum. Hindfemur not angulate (Pl. II, fig. 6). Hindcoxa pear-shaped, the hind edge gradually sloping (Pl. II, fig. 15), the comb on the inside situated near the apex. Apex of pleura of prosternite obtuse. Metasternum rotundate in front. First abdominal tergite with one row of bristles. The bristle of the metathoracic sternum situated close to the meral suture. The bristles placed posteriorly near the apex of the hindcoxa short and stout. To this group belong species No. 15—17.

#### (15) *Loemopsylla creusae* Rothsch. (1904).

(Pl. II, fig. 11; IV, fig. 12.)

*Pulex creusae* Rothschild (1904 a, p. 608, n. 4, t. 8, fig. 18, t. 9, fig. 25, Deelfontein, off *Felis caracal*); Baker (1905 a, p. 141).

A deeply coloured species with short rostrum.

**Head.** The rostrum (Pl. II, fig. 11) of the ♂ is considerably shorter than the maxillary palpus, while in the ♀ it equals the palpus in length. The proportional lengths of the segments of the maxillary palpus are 9, 16, 10, 20 in the ♂, and 10, 22, 11, 19 in the ♀. The grooves of insertion for the small hairs which are dispersed over the dorsal and lateral surfaces of the head appear as very conspicuous pale dots in the dark chitin. The occiput bears 3 subapical bristles on each side, besides the one which is placed dorsally close to the mesial line, and which is very small in the ♀, the first and second subapical bristles being separated by a wide interspace. There is no long bristle above the antennal groove between the centre and the ventral subapical bristle, the postmedian bristle as well as the one which, in other species, is situated behind the base of the antennal groove, being replaced by minute hairs. The first antennal segment of the ♂ bears 3 hairs at the hind edge and a transverse row of about 6 near the apex; in the ♀ there are 2 hairs at the tip of the apical projection.

**Thorax.** The pronotum bears a row of 10 to 12 bristles, the mesonotum a row of 8 or 9, and the metanotum one of 6 or 7 in the ♂ and 7 or 8 in the ♀, on the two sides together. The apex of the pleura of the prosternite is obtuse. The mesosternite bears 4 strong bristles, 2 of them standing close together above the stigma. The epimerum of the metathorax has a posterior row of 4 bristles in the ♂, and of 5 in the ♀, there being an additional bristle lower down on the sclerite representing the anterior row of other species of *Loemopsylla*. The apex of the metasternum is rounded.

**Abdomen.** The first tergite bears a row of 4 bristles on the two sides together. The second to seventh tergites have in the ♂ one bristle below the stigma and 4 (segment 6 and 7) or 6 (segment 2 to 5) on the back, there being a wide interspace between the infrastigmatal bristle and the dorsal ones. In the ♀ there are on the second to sixth segments 8 bristles, the gap between the two lower ones being much less wide than in the ♂, the second tergite bearing occasionally even 9 bristles. On the sternites the numbers of the bristles are in the ♂ 2, 4, 4, 4, 4 and 6; in the ♀ 2, 4 or 5, 5 or 6, 5 or 6, 6 and 7 to 9. The subapical bristle of the seventh tergite is short in both sexes.

**Legs.** The forecoxa bears 15 or 16 bristles. The comb on the pear-shaped hindcoxa is very variable, the number of spines being 3 to 10; the row is very irregular. There are 3 stout bristles posteriorly near the apex of the hindcoxa. The midfemur bears 2 subapical bristles on the outside and a row of 4 or 5 on the inside, the numbers being on the hindfemur 2 and 5 to 7 respectively. The tibiae have only one hair (or none) on the anterior surface, besides the apical and subapical bristles. The mid- and hindtibiae bear 6 pairs of dorsal bristles placed in notches (the apical bristles not being included in this number), the third pair being represented by a short stout bristle and a small hair. There is a regular lateral row of 7 or 8 bristles on the hindtibia. The longest ventral apical bristle of the hindtibia extends to the apex of the first tarsal segment, while the longest dorsal apical bristle reaches only to the subapical notch of the segment. The first and second hindtarsal segments are together as long as the hindtibia. The long bristles of the hindtarsus become very thin towards their apex, while the short apical bristles are stout and blunt. The long apical bristle of the second segment reaches beyond the base of the fifth. There is a long thin bristle at the apex of the fourth which extends to the claw. The fourth hindtarsal segment is nearly as broad as it is long. The fifth segment is large in all tarsi, distinctly widening apicad. The

lateral bristles of the same are long, those of the third pair standing close together. Ventrally at the apex this segment bears in all the tarsi one long and one short spine-like bristle.

**Modified segments.** ♂. The eighth sternite bears 4 or 5 small bristles on each side, besides one or two minute hairs. The clasper has two free processes, both being slender (Pl. IV, fig. 12). Beneath them there is a third short, broad process, which is not separated from the body of the clasper. The ninth sternite is somewhat acuminate, as shown in the figure, being elongate boat-shaped in side-view.—♀. The upper posterior corner of the eighth tergite is somewhat curved upwards, more distinctly so than in the ♂. The apical lateral margin is rounded. There is, on the outside, a row of about 15 bristles along the margin, besides 5 to 7 lateral bristles, 3 of the apical bristles being very stout; on the inside there are 5 or 6 apical bristles. The stylet is only a little longer than it is broad.

**Length.** ♂ 1.6 mm., ♀ 2.3 mm.

We have a large series of both sexes from Deelfontein, Cape Colony, off *Felis caracal*, March 1902, *Procapra capensis*, April 1902, and *Spreo bicolor*, May 1902, collected by C. H. B. Grant; also from Wakkerstroom, Transvaal, April 1904, off *Procapra capensis*, secured by the same collector.

(16) *Loemopsylla isidis* Rothsch. (1903).

(Pl. II, fig. 16; IV, fig. 11; VI, fig. 3.)

*Pulex isidis* Rothschild (1903, p. 313, n. 2, t. 5, figs. 2, 5, 6, 8, Harar, off *Procapra*); Wagner (1903, p. 508); Baker (1905 a, p. 142).

This insect, which agrees in size with *L. creusae*, is otherwise closely allied to *L. divergens*. It is much paler than both. The rostrum is longer than the maxillary palpus, reaching to the apex of the forecoxa. There is a bristle above the centre of the antennal groove on the occiput, this bristle being usually small in the ♀, while it is rather long and strong in the ♂. The subapical row of bristles of the occiput contains 5 bristles on each side, there being no wide gap between the first and second, as is the case in both *creusae* and *divergens*. The dorsal margin of the eighth abdominal tergite and the apical dorsal angle of the seventh are much less chitinated and project much less than in the two species mentioned. The number of bristles of the second to sixth tergites is 10 on the two sides together. The sternites bear about the same number of bristles as in *creusae*. The hindcoxa (Pl. II, fig. 16) has



only 2 bristles posteriorly at the apex. The upper process of the clasper is decidedly longer than in both the other species (Pl. IV, fig. 11). The eighth tergite of the ♀ (Pl. VI, fig. 3) bears along the margin about as many bristles as *creusae*, but these bristles are of more even size, the upper 3 or 4 not being much thicker than the following ones. The stylet of the ♀ is about twice as long as it is broad at the base.

A series of both sexes was received from Harar, taken off *Procavia erlangeri* on March 12, 1900 (Baron Carlo von Erlanger and Oscar Neumann).

(17) *Loemopsylla divergens spec. nov.*

(Pl. II, fig. 10 ; VI, fig. 2.)

Closely resembling *L. creusae*, with which it occurs together. The species is larger than *creusae*. The rostrum is much longer than the maxillary palpus and reaches to the apex of the forecoxa, the last segment (Pl. II, fig. 10) being half as long again as in *creusae*. The occiput bears a bristle above the centre of the antennal groove. The small hairs placed in the ♂ along the antennal groove are more numerous than in *creusae*. The second to sixth abdominal tergites bear 5 bristles on each side in the ♀, the interspace between the first and second bristle being smaller than the one between the second and third. In the ♂, however, the sixth tergite bears only 3 or 4 bristles on each side, the two lower ones being placed wide apart. The dorsal apical angle of the seventh and eighth tergites of the ♀ is more strongly curved upwards than in the ♀ of *creusae*. The sternites of segments 4 to 7, especially in the ♀, bear a few more bristles than in *creusae*. The genitalia of the ♂ are nearly the same as in *creusae*, but the two claw-like projections which protrude externally from the penis are larger and more strongly chitinized. The bristles along the edge of the eighth tergite of the ♀ (Pl. VI, fig. 2) are more numerous than in *creusae*. The stylet of the ♀ is almost thrice as long as it is broad at the base, being much longer than in *creusae*.

We have a series of both sexes, taken off *Procavia capensis*, April 1902, and off *Felis caracal*, March 1902, by C. H. B. Grant, at Deelfontein, Cape Colony. The present insect is perhaps a larger southern race of *L. isidis*.

## 4. Group of species.

Episternum of metathorax separated from the sternum. Hindfemur not angulate beneath near the base. Hindcoxa broader than in the three previous groups, posteriorly with two slender subapical bristles. Stigma of the proximal abdominal segments hardly larger than the groove for insertion of the first bristle of the tergites. Ventral angle of metasternum acuminate. To this group belong Species No. 17—21.

(18) *Loemopsylla erilli* Rothsch. (1904).

(Pl. II, figs. 6, 15; V, fig. 2; VII, fig. 5.)

*Pulex erilli* Rothschild (1904 a, p. 610, n. 5, t. 8, figs. 16 and 17, t. 9, fig. 22, Deelfontein, off *Zorilla striata*, *Suricata tetradactyla*, *Xerus capensis*).

Easily distinguished from the other species of *Loemopsylla* which have the hindfemur non-angulate by the long maxillary and labial palpi.

**Head.** The maxillary palpus reaches to the apex of the forecoxa, while the rostrum extends beyond the trochanter, being especially long in the ♀. The subapical row of bristles of the occiput contains 4 bristles on each side, the first and second being placed wide apart. There are about 30 small hairs above the antennal groove in the ♂. The first antennal segment of the ♂ is long.

**Thorax.** The bristles on the three tergites on the two sides together, are in the ♂ 12, 10, 8 or 9, and in the ♀ 12, 11 to 13, 10 or 11. The mesopleura bears 3 bristles. In the ♀ the epimerum of the metathorax has two rows of bristles (3, 4 or 5), the anterior row being represented in the ♂ by one bristle and the second by 3 to 5.

**Abdomen.** The first tergite bears two rows of bristles, the first row containing, on the two sides together, in the ♂ 2 or 3 bristles and the second row 4, the numbers being in the ♀ 3 or 4 and 5 respectively. On the second to seventh tergites there are on the two sides together the following numbers of bristles: ♂, 10 on tergites 2 to 6, and 6 or 7 on the seventh; ♀, 10 to 13, 11 or 12, 12, 10 or 11, 10 or 11, and on the seventh 7 or 8. The sternites bear in the ♂ 4 bristles, the first sternite having only 2, on the two sides together. In the ♀ the basal sternite bears 2 ventral bristles and on each lateral surface 4 or 5 more, the numbers on the next 5 sternites being 8 to 16, 6 to 9, 6, 6, and 4 respectively, on both sides together; the small number of bristles on the seventh sternite being a very remarkable character. The subapical bristle of the seventh tergite is shorter than the second midtarsal segment.

**Legs.** The forecoxa bears about 15 bristles. The hindcoxa is rather narrow, being in the ♂ half as long again as it is wide and bearing a comb of 7 to 9 spines. The sinus of the hindcoxa (Pl. II, fig. 15) is extremely shallow. Both the mid- and hindcoxae bear posteriorly 2 subapical bristles. The midfemur has exteriorly 1 or 2 subapical bristles and on the inner side a subventral row of 4, the numbers being on the hindfemur (Pl. II, fig. 6) 1 or 2 and 6 respectively. The hindtibia bears a lateral row of 4 to 6 bristles. The long apical bristles of this tibia are stout in the ♀, the ventral one extending to the apex of the first hindtarsal segment, while the dorsal one hardly reaches the subapical notch of this segment, both bristles being more slender and longer in the ♂. The longest apical bristle of the second hindtarsal segment in the ♂ reaches almost to the apex of the fifth segment, while in the ♀ it extends beyond the middle of the segment. The first hindtarsal segment has no bristles on the external surface, other than the bristles which are placed at the edges. The fifth hindtarsal segment is as long as the second midtarsal segment. In the fore- and midtarsi the fifth segment bears in the ♂ 4 short stout ventral apical bristles, 3 being placed at the apical edge and 1 proximally to them; no other species of the present group has 4 such bristles. In the hindtarsus of the ♂ and in all tarsi of the ♀ the fifth segment has 2 such bristles only, one being short and the other long.

**Modified segments.** ♂. There are 3 ventral bristles on each side of the eighth sternite, besides a very few minute hairs. The clasper (Pl. V, fig. 2) bears a long slender free process which has several long bristles at and near the apex, besides several smaller ones. A second process is conical, while a third is broad and short, being excavate on its upper surface. The ninth sternite (ix. st.) has a few minute hairs at the tip and one longer one proximally to the apex.—♀. The eighth tergite (Pl. VII, fig. 5) has an apical row of usually 6, sometimes 7 or 8, bristles and on the lateral surface 2 more bristles; on the inner side there is an apical row of 4 bristles and 1 or 2 minute hairs. The anal sternite is obliquely truncate. The stylet is more than twice as long as it is broad.

**Length.** ♂ 1.6 mm., ♀ 2.2 mm.

We have a series of this species from Deelfontein, Cape Colony, collected by C. H. B. Grant, as follows: *Zorilla striata*, August 1902; *Suricata tetradactyla*, April 1902; *Xerus capensis*, April 1902.

(19) *Loemopsylla mycerini* Rothschild (1904).

(Pl. III, fig. 2; VII, fig. 2.)

*Pulex mycerini* Rothschild (1904 *b*, p. 1, n. 1, t. 1, figs. 1, 2, 4, Lower Egypt, off *Gerbillus tarabuli*); Baker (1905 *a*, p. 142).

**Head.** The rostrum is somewhat longer than the maxillary palpus, but does not reach to the apex of the forecoxa. The second segment of the maxillary palpus is shorter than the fifth, being one-fifth longer than the first. The subapical row of bristles of the occiput contains 4 bristles on each side, besides a dorsal one, the first and second bristles being placed wide apart. Beneath the first bristle there is a rather long thin hair. Above the centre of the antennal groove there is one long bristle.

**Thorax.** The pronotum bears 12 bristles, the mesonotum 12 in the ♂ and 10 or 11 in the ♀, the metanotum 8 or 9 in the ♂ and 10 in the ♀. The pleura of the mesothorax has 4 bristles, two of them placed close together above the stigma. On both the sternum and the episternum of the metathorax there is a single bristle, while the epimerum bears two rows of 6 to 8 bristles (2 to 4, 4). The pleura of the prosternite is pointed behind.

**Abdomen.** The first tergite bears two rows of bristles, the first row (which is sometimes missing in the ♀) containing 3 to 5, and the second 4 to 7 bristles. The second to fourth tergites in the ♂ bear 12 bristles on the two sides together and the fifth to seventh tergites 10 bristles; in the ♀ the second to sixth have 12 bristles and the seventh 10. The subapical bristle of the seventh tergite is much longer than the second midtarsal segment, equalling the second hindtarsal segment. The basal sternite bears in both sexes 2 ventral bristles on the two sides together, the following 4 sternites having in the ♂ 4 bristles and in the ♀ 6 to 8, the sternite of the seventh segment bearing 6 bristles in the ♂ and 8 in the ♀.

**Legs.** The forecoxa bears about 20 bristles. The hindcoxa is rounded posteriorly, the sinus being very shallow. There are 2 subapical bristles on the hinder side. The comb contains 6 or 7 spines. The hindfemur is broad near the base, being strongly rounded ventrally. It has a row of 5 to 7 bristles on the inside and 2, in the ♀ sometimes 3, subapical bristles on the outside. The longest ventral apical bristle of the hindtibia is a little shorter than the dorsal one, the latter reaching close to the apex of the first hindtarsal segment (Pl. III, fig. 2).

The second hindtarsal segment bears two long apical bristles on the posterior side, the longer one extending nearly to the apex of the fifth segment, while the other bristle only reaches to the centre of the segment. The fifth segment is only twice as long as it is broad near the apex, being but slightly dilated distally. There are in both sexes 2 ventral apical bristles on this segment.

**Modified segments.** ♂. These segments (Pl. VII, fig. 2) closely resemble those of *L. regis*. The eighth sternite bears 3 bristles on each side, one being lateral and two ventral, the second ventral bristle being placed close to the apex of the segment. The clasper has 3 processes. The first process is slightly widened distally and provided with an apical row of bristles; the second process is very slender, while the third is short and broad. The ninth sternite has a few minute hairs at and near the apex and two longer ones on the side.—♀. The eighth tergite has on the outside an apical row of 7 or 8 bristles, and 3 or 4 bristles on the lateral surface, while there is an apical row of 7 or 8 bristles on the inside. The stylet is about twice as long as it is broad near the base. The anal sternite is truncate, the apex being narrower (in side-view) than in *L. erilli*.

**Length.** ♂ 1.5 mm., ♀ 2 mm.

This species is known to us from Bir Victoria, Natron Valley, Lower Egypt, where the junior author took a series of it off *Gerbillus tarabuli* and *Pachyuromys duprasi natronensis*, in March 1903.

(20) *Loemopsylla gerbilli* Wagn. (1903).

(Pl. III, fig. 1; VII, fig. 1.)

*Pulex pallidus* Wagner (1894, p. 440, non Taschenberg 1880, err. ident.).

*Pulex gerbilli* Wagner (1903, p. 309, Transcaspia, off *Gerbillus*); Baker (1905a, p. 141).

Very close to *L. mycerini*. The insect differs from *mycerini* especially in the bristles of the tibiae and tarsi being considerably longer. The first hindtarsal segment, for instance, bears 5 apical bristles which reach to the apex of the second segment or beyond (Pl. III, fig. 1), and the second segment has 3 very long apical bristles, one extending beyond the apex of the fifth segment, another reaching to the apex and the third to the centre of the fifth segment. The tarsi, too, are much longer. The two free processes of the clasper (♂), moreover, are separated from each other (Pl. VII, fig. 1), and the third process is longer than in *mycerini*. The eighth sternite of the ♂ bears a ventral row of 4 bristles on each side.

We have only examined two specimens received from Dr J. Wagner, taken off *Gerbillus spec.* at Tedschen, Transcaspia, in July 1893.

(21) *Loemopsylla ramesis* Rothsch. (1904).

(Pl. V, fig. 6.)

*Pulex ramesis* Rothschild (1904 b, p. 2, n. 2, t. 1, fig. 3, Lower Egypt, off *Pachyuromys duprasi natronensis* and *Gerbillus tarabuli*); Baker (1905 a, p. 143).

Likewise closely related to *L. mycerini*. The rostrum, however, is only as long as the maxillary palpus, reaching to the apical third or fourth of the forecoxa, and the genitalia of the ♂ are very distinctive (Pl. V, fig. 6). The eighth abdominal sternite of the ♂ bears 2 long lateral bristles and a row of 2 to 4 ventral ones. The upper free process of the clasper is broad, truncate, bearing several long bristles at the apex and a number of shorter ones on the outer and upper sides. The third process is also broader and longer than in *mycerini*.

A small series of both sexes from Bir Victoria, Natron Valley, Lower Egypt, off *Pachyuromys duprasi natronensis* and *Gerbillus tarabuli*, March 1903, collected by N. C. Rothschild and F. R. Henley.

(22) *Loemopsylla regis* Rothsch. (1903).

(Pl. II, fig. 14; IV, fig. 10; V, fig. 7.)

*Pulex regis* Rothschild (1903, p. 312, n. 1, t. 5, figs. 1, 3, 4, 7, 9, S. Arabia, off *Meriones rex*); Wagner (1903, p. 508); Baker (1905 a, p. 143).

The numerous long and slender bristles situated on the thorax and abdomen distinguish this insect markedly from all the others of the present group of species.

**Head.** The rostrum in the ♂ is about as long as the maxillary palpus, reaching to the apex of the forecoxa, while in the ♀ it is longer than the palpus. The subapical row of bristles of the occiput contains on each side 6 to 8 bristles, there being also a long bristle above the centre of the antennal groove.

**Thorax.** The tergites bear, on the two sides together, a row of 18 or 19 bristles, there being some additional bristles on the back of the pro- and mesonotum in front of the row in the ♂. The pleura of the prosternite is pointed posteriorly. The mesopleura bears 5 or 6 bristles. There are 2 bristles on the episternum of the metathorax and 13 to 15 on the epimerum.

**Abdomen.** The first tergite has an irregular median row of 10 bristles and a postmedian row of 6 to 8 in the ♂, and 10 in the ♀, on both sides together, there being some additional bristles on the back. The numbers of bristles on the second to seventh tergites are in the ♂ 20, 20, 17 to 19, 17, 15 to 17, 14, and in the ♀ 22, 20 to 22, 20 to 22, 19 to 21, 19 or 20, 16 or 17. The subapical bristle of the seventh tergite is considerably longer than the second midtarsal segment. The bristles on the sternites are in the ♂ 2 on the basal one and 4 on the others, in the ♀ also 2 on the basal sternite and 6 to 8 on the others.

**Legs.** The forecoxa bears about 30 bristles. The hindcoxa (Pl. II, fig. 14) is much more rounded than in the allied species, bearing posteriorly 2 subapical bristles. The bristles of the tibiae and hindtarsi are long. The longest apical bristle of the second hindtarsal segment reaches far beyond the apex of the fifth segment, a second bristle on the same side extending to the apex of this segment, while a bristle on the anterior side of the second segment reaches at least to the centre of the fifth. This latter segment, in the ♂, is as long as the second midtarsal one, while in the ♀ it is shorter than that segment.

**Modified segments.** ♂. The eighth sternite bears one long bristle on the lateral surface and 2 to 4 along the ventral margin. The clasper (Pl. IV, fig. 10) has two free processes, one being long and rather broad, bearing a number of long bristles, the other being slender. The ninth sternite resembles that of *L. mycerini*.—♀. The eighth tergite (Pl. V, fig. 7) has an apical row of 7 to 9 bristles on the outside and of 7 or 8 on the inside, there being also from 3 to 5 lateral bristles on the outer surface. The anal sternite is acuminate.

**Length.** ♂ 1.3 mm., ♀ 1.8 mm.

We have specimens of both sexes from Lahaj, north of Aden, off *Meriones rex*, collected by Baron Carlo von Erlanger and O. Neumann on 21st December, 1899.

### (23) *Loemopsylla conformis* Wagn. (1903).

*Pulex pallidus* Wagner (1894, p. 440, non Taschenberg, 1880, err. ident.).

*Pulex conformis* Wagner (1903, p. 508, n. 2, Transcaspia, off a small owl); Baker (1905 a, p. 141).

This insect is not known to us from specimens. It possibly may be the same as *L. ramesis*. The chief characters given by Dr Wagner are as follows:

The rostrum falls considerably short of reaching to the apex of the forecoxa. The lower angle of the eighth abdominal tergite of the ♀ is

not produced rectangularly. The eighth abdominal sternite of the ♂ bears 2 ventral bristles on each side, placed far apart from each other. The two movable processes of the clasper of the ♂ are larger than in *L. gerbilli*.

Dr J. Wagner received one pair of this insect from Sultan Bent, River Mourgab, Transcaspia, off a small species of owl.

### 5. Group of species.

Episternum of metathorax separated from the sternum. Hindfemur not angulate beneath, its greatest width further back than in all the other forms of *Loemopsylla*. Bristles of body and legs stout and black. Frontal portion of head with 6 long bristles; occiput with about 8 on each side, arranged in two oblique rows, besides the subapical row. Stigmata of abdomen longer than the groove for insertion of the first bristle of the tergite; in ♂ at least 2 bristles beneath the stigmata, in ♀ 4 or more. Midcoxa very strongly rounded behind, the hind edge being almost semicircular. To this group only species No. 24 belongs.

#### (24) *Loemopsylla chephrenis* Rothsch. (1903).

(Pl. II, figs. 5 and 7.)

*Pulex chephrenis* Rothschild (1903, p. 86, n. 5, t. 1, fig. 7, t. 2, figs. 14 and 18, Cairo, off *Acomys cahirinus* and *Dipus jaculus*); Baker (1905 a, p. 141).

*Pulex alternans* Wahlgren (1904, p. 1, figs. 1 and 2, Cairo, off *Acomys cahirinus*); Baker (1905 a, p. 140).

The most distinct looking species in the genus on account of the very dark coloured bristles.

**Head.** The frons (Pl. II, fig. 5) is more strongly and abruptly rounded, especially in the ♀, than in the other species of *Loemopsylla*. There are 6 long strong bristles on the frontal portion of the head. One of these bristles is placed in front of the eye, but at a distance from it, one above the insertion of the palpus, a third at the oral edge, a fourth beneath the eye and two more are situated on the genal process. On the occiput there is a subapical row of 7 strong bristles and two oblique lateral rows, the anterior one consisting of 3 bristles and the second one of 4. The ♂ has 5 to 8 small hairs above the antennal groove. The eye is small. The posterior apical angle of the first antennal segment of the ♀ is hardly produced at all. The second segment, in both sexes, bears a transverse row of 3 hairs, the third hair being long, while the others are much shorter than in the other species of *Loemopsylla*, there being also 2 short thin hairs at the hind edge of the segment. In the ♀ this segment differs from that of other species in the apical,



narrowed, portion being longer. The rostrum is longer than the maxillary palpi, reaching to the apical third of the forecoxa.

**Thorax.** The three thoracical tergites bear, on the two sides together, 14, 10 or 12, and 12 bristles respectively. There are 6 bristles on the pleura of the mesothorax, while the epimerum of the metathorax bears 10 or 11 bristles in two rows (5 to 7, 4 or 5).

**Abdomen.** The first tergite bears two rows of bristles, the first row consisting of 5 to 7, and the second of 6 bristles. The rows of bristles on the second to sixth tergites extend much further down the sides than in the other species of *Loemopsylla*, their number being in the ♂ 14 to 16, in the ♀ 18 to 20, on the two sides together, the seventh tergite having 10 to 11 bristles. The dorsal subapical bristle of this tergite is longer than the first and second midtarsal segments together. On the sternites there are in the ♂ 4 bristles, in the ♀ 8, the basal sternite having only 2 bristles in both sexes. The ventral line of this sternite is, in side-view, more oblique proximally than in the other species, being rather sharply curved near the apex.

**Legs.** The forecoxa bears about 30 bristles. The midcoxa is very strongly rounded behind, being almost as broad as it is long. The hindcoxa is likewise rounded and broad, bearing a comb of 4 to 6 spines. The mid- and hindfemora have on the outside 2 (occasionally 3) ventral subapical bristles and one lateral subapical one, there being only one bristle on the inside, the usual lateral row of bristles being absent. The hindfemur, moreover, has a very characteristic shape (Pl. II, fig. 7), its widest point lying further back than in the other species of *Loemopsylla*. The hindtibia bears a lateral row of 6 to 7 bristles and 2 additional ones in between this row and the dorsal bristles. The tarsi bear more bristles on the outer surface than in the other species of *Loemopsylla*. The longest apical bristle of the second hindtarsal segment reaches just beyond the apex of the fourth segment. While the first hindtarsal segment of the other species of *Loemopsylla* bears 5 to 7 bristles close together on the inside at the apex (see Pl. I), in *chephrenis* one bristle only exists in their place. The first pair of lateral bristles of the fifth foretarsal segment is placed nearer the mesial line than the other bristles. This segment bears in the ♂ 3 short stout apical ventral bristles; in the ♀ it has 2 apical bristles instead, one on each side, both being rather thin, the fifth segment of the mid- and hindtarsi bearing in both sexes only 2 such bristles.

**Modified segments.** ♂. The eighth sternite bears on each side one lateral and two ventral bristles. The clasper has two free slender pro-

cesses, the smaller one bearing one long apical bristle and some shorter ones. The manubrium is slightly club-shaped. The ninth sternite is canoe-shaped in side-view, narrowing rather strongly basally.—♀. The eighth tergite has, on the outside, 3 long lateral bristles and a subapical row of 5 to 7, there being 4 to 6 short apical bristles on the inside.

**Length.** ♂ 1.4 mm., ♀ 2.2 mm.

We have a small series of both sexes from Cairo, Egypt, off *Acomys cahirinus* and *Jaculus jaculus*<sup>1</sup>, taken in January and March, 1901, by N. C. Rothschild and A. F. R. Wollaston.

We also received a pair of cotypes of *alternans* Wahlgr. from the Riksmuseum at Stockholm.

#### 5. Genus: *Rhopalopsyllus* Baker (1905).

*Rhopalopsyllus* Baker (1905 a, p. 128, type of name: *lutzi*; descript. erroneous); Rothschild (1906, p. 173).

The short description which Baker gave of his genus *Rhopalopsyllus* is erroneous and insufficient. From the incidental statement, however, on page 130 of the treatise quoted above that "the upper edge of the antennal groove usually has a row of many short and thick, but minute, spines or teeth," we may safely conclude that the name *Rhopalopsyllus* was meant to apply as a generic term to the species characterised below.

**Head.** Frons always with notch (Pl. II, fig. 3). Antennal groove extended to the vertex in both sexes, widely open behind, the genal process being short (Pl. II, fig. 3). First segment of the antennae long in ♂ and ♀; second almost square in side-view, bearing several bristles at the hinder edge, the last bristle being the longest and stoutest; club a little longer in ♂ than in ♀, segmented all round, the segments deeply separated on hinder side, the first elongate-ovate, the next three lamelliform in lateral aspect, separate, very oblique in position, inclining apicad (Pl. II, fig. 3). In both sexes a row of short, stout, spine-like hairs along the antennal groove, standing close together, the row extending from the hinder edge of the head to the central row of bristles of the occiput. Occiput with at least one row of bristles besides the subapical row. Labial palpus consisting of 5 to 7 segments; the segmentation very distinct all round; tip of last segment excised, bearing 3 small hairs on each side (Pl. II, fig. 9). There are 3 bristles

<sup>1</sup> The specimen of *Jaculus jaculus* from which a *L. chephrenis* was taken had been kept in captivity for two days in a cage with a live *Acomys cahirinus*. This flea is probably confined to *A. cahirinus*.

in front of the eye and usually another row farther frontad. One or more bristles beneath the eye.

**Thorax.** Upper edge of prosternum subangulate before middle (Pl. II, fig. 3). Pronotum with two rows of bristles, meso- and metanotum with three or more rows. Mesonotum without subapical spines. Metanotum as well as the anterior abdominal tergites bearing a comb of very small teeth at the apical edges. Epimerum of mesothorax more or less completely covering the stigma. Episternum of metathorax large, more than half the size of the sternum (side-view), bearing two long bristles, one at upper margin and one at lower, besides some small hairs. Sternum of metathorax with one short bristle. Epimerum of metathorax with two rows of bristles, the anterior row being situated at some distance from the stigma, while the second row extends from the stigma downwards.

**Abdomen.** Tergites with at least two rows of bristles, but the anterior row sometimes represented by a few dorsal bristles only; seventh tergite bearing a long bristle at the apical edge, placed on a cone, the edge of the segment being here sinuate, projecting backwards dorsally in between the long bristles (Pl. VI, fig. 7). Basal sternite in ♂ and ♀ with small hairs on the side. Stigmata large. Anal segment large, especially the sternite.

**Legs.** Internal rod-like incrassation of midcoxa dividing about the centre, at any rate farther from the base than in *Pulex* and *Loemopsylla*. No comb on hindcoxa. First segment of fore- and midtarsi shorter than the second. Hindfemur bearing at least one row of bristles on the outer surface. Fifth tarsal segment (Pl. III, fig. 10) with 4 lateral bristles besides the subapical hair, and with a row of 2 to 4 small hairs on the ventral surface.

**Modified segments.** ♂. Clasper large, triangular, bearing a long finger-like movable process (Pl. VI, fig. 7, F). Ninth sternite boomerang-shaped, the inner vertical portion extending upwards beyond the manubrium of the clasper and being widened at apex.—♀. Eighth tergite always with some bristles above the stigma (Pl. VII, fig. 10).

The genus is purely American. It contains at present twelve species, one of them (*lutzi*) being doubtfully distinct from *cleophontis*.

**Key to the species of *Rhopalopsyllus*.**

- a. Labial palpus consisting of 6 or 7 segments .....b
- Labial palpus consisting of 5 segments .....c
- b. Metanotum with 3 rows of bristles .....Species No. 1
- Metanotum with 2 rows of bristles .....Species No. 2
- c. Rostrum extending beyond the trochanter .....Species No. 10
- Rostrum extending to the apex of the forecoxa or being shorter .....d
- d. Occiput with 2 rows of bristles .....Species No. 11
- Occiput with 3 rows of bristles .....e
- e. Anterior edge of pygidial plate much raised, projecting; fifth segment of all tarsi long, dilated towards apex, that of foretarsus larger than the second midtarsal segment.....f
- Anterior edge of pygidial plate slightly raised; fifth tarsal segment short, in hindtarsus about as long as the second midtarsal segment .....h
- f. Basal abdominal sternite with 2 lateral rows of small bristles .....g
- Basal abdominal sternite with 3 or 4 irregular lateral rows of small bristles. Species No. 5
- g. Hindtibia with 2 or 3 bristles on the inner surface .....Species No. 3
- Hindtibia with 4 or 5 bristles on the inner surface .....Species No. 4
- h. Midtibia with numerous bristles scattered over the outer surface as on the foretibia. Clasper of ♂ with 3 long bristles below the tip .....i
- Midtibia with one lateral row of bristles on the out- and inside. Clasper of ♂ with 2 bristles below the tip.....Species No. 12
- i. Hindcoxa with 2 bristles posteriorly near the apex; the long bristle situated in the fourth notch of the hindtibia extending beyond the apex of the tibia. Species No. 9
- Hindcoxa with 3 bristles posteriorly near the apex .....k
- k. Metanotum with 4 rows of bristles, the anterior row consisting of about 6 bristles .....Species No. 6
- This fourth row of bristles of the metanotum absent, or represented by 1 or 2 bristles only .....l
- l. The bristles situated below the tip of the ninth sternite (♂) very numerous; movable process of clasper short .....Species No. 8
- The bristles situated below the apex of the ninth sternite (♂) more evenly distributed; movable process of clasper long and slender .....Species No. 7

**(1) *Rhopalopsyllus cleophontis* Rothsch. (1904).**

(Pl. II, figs. 3, 9; III, fig. 10; VI, fig. 7.)

*Pulex cleophontis* Rothschild (1904 a, p. 614, n. 9, t. 10, fig. 32, Buenos Ayres, off *Muletia septemcincta*).*Rhopalopsyllus cleophontis* Baker (1905 a, pp. 130, 143).

The largest known species of this genus, apart from *R. lutzi*. The bristles on the body are somewhat thin for the size of the insect.

**Head.** The labial palpus has six segments, sometimes one or the other segment being again divided on one side. The basal segment (= labium) of the rostrum is very short. The anterior row of bristles of the frons (Pl. II, fig. 3) is represented by 3 or 4 thin bristles (in the original description it was erroneously stated that this anterior row is absent). The bristle situated at the apex of the second antennal segment is very thick.

**Thorax.** The pro- and mesonotum bear each two rows of bristles, the posterior row consisting of 12 or 13 bristles. On the metanotum there are three rows. The pleura of the mesothorax bears 4 bristles, while the epimerum of the metathorax has 10 or 11 bristles in two rows (4 to 6, 4 to 6).

**Abdomen.** The first abdominal tergite has a comb of short apical spines like the metanotum, the second and third, and sometimes the fourth and fifth tergites also bearing some apical spines. The first tergite has two rows of bristles, the anterior row consisting of about 12, the posterior row of 8 bristles. The second to seventh tergites have a posterior row of 12 bristles, one anterior row being represented in the ♂ on the second tergite by 4 or 5 bristles, on the two sides together, and by 2 to 4 bristles on the other tergites, while the numbers are somewhat increased on the proximal tergites of the ♀, there being in this sex also a bristle just beneath the stigma. The basal sternite bears two lateral rows of hairs, besides some ventral bristles. The second sternite has a row of about 10 bristles and one or two additional bristles in front of this row, the number of bristles being smaller on the sternites of the fourth to sixth segments. In the sternite of the seventh segment there is a row of 7 to 10 bristles, the ♀ having ventrally 2 or 3 bristles in front of this row.

**Legs.** The forecoxa bears more than 30 bristles, the bristles being closer together near the base than near the apex of the coxa. The midcoxa has bristles on both sides along the anterior edge from the base to the apex. On the inside of the hindcoxa there is a row of bristles along the anterior edge, the bristles being closer together towards the apex than towards the base of the coxa. There are 3 bristles posteriorly at the apex of the hindcoxa, and 2 on the midcoxa. The hindfemur bears on the inside a row of 8 or 9 bristles, there being on the outside 2 subapical ventral bristles and 2 or 3 anterior lateral ones, besides a few dorso-lateral hairs. The hindtibia bears 9 bristles on the outer side, besides about 12 shorter ones which stand at and near the anterior edge of the tibia. The tarsi and their bristles are

very robust, the fifth segment (Pl. III, fig. 10) especially being long and broad. In the foretarsus this segment is as long as the four other segments together, its lateral bristles as well as the claws being long and strong. The second midtarsal segment is nearly twice the length of the first. The proportional lengths of the mid- and hindtarsal segments are:

Segment		First	Second	Third	Fourth	Fifth
Midtarsus:	♂	18	31	18	9	40
	♀	19	37	20	12	48
Hindtarsus:	♂	50	44	27	14	46
	♀	60	50	30	14	50

**Modified segments.** ♂. The eighth sternite bears a row of about 12 bristles on both sides together, the ventral bristles of this row being smaller than the lateral ones, there being also some small hairs in front of the row. The clasper is triangular (Pl. VI, fig. 7), with the apex rounded off. There are 3 long stout bristles close together below the apex and a thinner and paler one above them at the apex, there being also a number of bristles and hairs along the dorsal edge of the clasper. The finger (F) is long and slender, almost reaching to the subapical bristles of the clasper. The finger bears 2 long thin bristles near the apex, besides a number of thin hairs. Where the finger joins the clasper there are 4 bristles (3, 1) at the ventral margin of the clasper. The manubrium of the clasper is slender. The ninth sternite (ix. st.) is elongate boat-shaped, bearing a number of bristles in the apical half along the ventral margin, two of them being somewhat thicker and longer than the others.—♀. There are 3 small bristles above the stigma of the eighth tergite on each side. From the stigma downwards this segment bears a row of 12 to 14 bristles, the row being widely interrupted beneath the centre. There is a row of about 12 bristles along the apical edge on the outside, five or six of the apical bristles being close together, and a row of about 12 on the inside. On the outside of the segment, near the base, there is a patch of 3 to 5 small bristles. The stylet is cylindrical, or nearly so, bearing a long apical bristle, but no subapical notch.

**Length.** ♂ 2.6 to 2.9 mm., ♀ 2.6 to 3.7 mm.<sup>1</sup>

We have 2 ♂♂ and 2 ♀♀ from the southern portion of the Province of Buenos Ayres, from *Muletia septemcincta*, collected by the late Dr

<sup>1</sup> Some of the mounted specimens are much extended, which partly accounts for the difference in length.

Carlos Berg; and several pairs from Minas Geraës (A. Kennedy), no host being given.

The large development of the fifth tarsal segment is suggestive of the genus *Malacopsylla* Weyenb.

We also have a ♂ and a ♀ from Sapucay, Paraguay, off *Didelphys spec.*, collected by W. Foster, the ♂ differing from the above described ♂♂ in the clasper bearing but 3 bristles at the tip.

(2) *Rhopalopsyllus lutzii* Baker (1904).

*Pulex lutzii* Baker (1904, pp. 378, 380, São Paulo, off *Grisson vittata*).

*Rhopalopsyllus lutzii* Baker (1905 a, pp. 128, 130).

We have not seen Dr Baker's specimens, and therefore must rely on the description he gives. Unfortunately the description appears to be very faulty, some of the statements at least being certainly incorrect. In 1904 the palpus is said to be "apparently six-jointed," while it is stated to be four-jointed in 1905.

The metanotum is described as having only two rows of bristles like the pro- and mesonotum, a statement which requires verification. Only the first and second abdominal tergites have the second row of smaller bristles, the remainder having each but a single row of about 13 bristles. The frontal portion of the head bears two rows of bristles, the anterior row consisting of 4 smaller bristles, the second row of 3 much larger ones. The clasper appears to be similar to that of *R. cleophontis*, being "armed at the tip with 3 stout close set spines." The stylet of the ♀ is cylindrical. The hindtibia has about 8 stout bristles on the outer side.

**Length.** ♂ 5.5 mm., ♀ 6 mm.

Both sexes from São Paulo, off *Grisson vittatus*, in the United States National Museum.

We believe this insect to be a near ally of *R. cleophontis*.

(3) *Rhopalopsyllus australis* Rothschild (1904).

(Pl. III, fig. 11; VI, figs. 10, 11.)

*Pulex australis* Rothschild (1904 a, p. 613, n. 8, t. 9, fig. 29, t. 10, figs. 34, 36, partim, Tabasco, Mexico, off *Dicotyles labiatus*).

*Rhopalopsyllus australis* Baker (1905, pp. 130, 143).

**Head.** The first segment of the maxillary palpus is as long as the third, the second being a very little shorter than the fourth. The

rostrum does not reach to the apex of the forecoxa. The labial palpus has five segments. The frons bears two rows of bristles, those of the anterior row being short and rather thin, except the one which is situated near the antennal groove. There are three rows of bristles on the occiput.

**Thorax.** The pronotum has two rows of bristles (8, 15), the mesonotum also two (13 to 16, 15), and the metanotum four rows (5 to 10, 20, 20, 15 to 17). There are 4 bristles on the pleura of the mesothorax, while the epimerum of the metathorax bears 10 to 12 bristles in two rows (5 or 7, 4 to 6), there being no bristles close to the upper anterior corner of this sclerite.

**Abdomen.** The first tergite has two rows of bristles (12 and 8 or 10); on the second tergite there are about 8 small bristles in the anterior row and 12 in the posterior series. The third to seventh tergites have in the ♂ from 0 to 3 bristles in the anterior series and 12 in the posterior, while the numbers in the ♀ are 4 and 14, the seventh tergite bearing in this sex a row of 9 or 10 bristles and in front of it about 7 small ones. On the basal sternite there are two lateral rows of hairs. The sternites of the third to seventh segments bear in the ♂ 4 long and some small bristles and in the ♀ 8 long bristles on the two sides together, the second sternite having some additional hairs in front of the row in both sexes. The first tergite, like the metanotum, has a comb of short apical spines, the following four tergites also bearing from 1 to 3 small spines. The edge of the pygidial plate is strongly raised anteriorly, projecting backwards in a side-view.

**Legs.** The hindcoxa has 3 bristles posteriorly at the apex. The femora bear two rows of bristles on the outside, the upper row of the hindfemur being restricted to the posterior half of the femur. The forefemur has on the inside only 2 small bristles, one situated nearly in the centre, the other ventrally near the apex. The hindfemur bears on the inside a row of 7 or 8 bristles. The bristles of the tibiae and hindtarsi are long and thick. The hindtibia bears about 12 short bristles at and near the anterior side, while there are usually 10 longer ones on the lateral surface. In the ♀ the longest apical bristle of the hindtibia reaches to the apex of the first hindtarsal segment, the longest bristle of this segment extending to the tip of the second segment, and the longest bristle of the second segment to the apex of the fourth, these bristles being somewhat longer in the ♂. The fifth tarsal segment is large, the claws being also very long, this segment of the hindtarsus being as long as the first and second segments of the midtarsus together (Pl. III, fig. 11).



The measurements of the mid- and hindtarsi together are :

Segments		First	Second	Third	Fourth	Fifth
Midtarsus :	♂	14	23	14	9	30
	♀	16	26	18	10	36
Hindtarsus :	♂	49	35	22	13	39
	♀	52	38	23	13	40

**Modified segments.** ♂. The eighth sternite bears a row of about 9 rather short bristles on the two sides together. The large clasper bears 2 stout bristles below the tip and a number of slender bristles at and near the upper edge. The movable "finger" is elongate-rectangular, the apex being slightly rounded. At the edge of the clasper near the base of the finger there are 3 thin bristles (2, 1). The external (ventral) portion of the ninth sternite (Pl. VI, fig. 11) gradually narrows towards the apex, bearing a number of slender bristles and hairs.—♀. The eighth tergite has 1 to 3 bristles above the stigma. There is a row of 4 long and 5 or 6 short bristles extending from the stigma downwards and an additional row of about 12 bristles along the apical edge on the outside, 4 of the apical bristles being long and placed close together. On the inside of the segment there are about 9 bristles. The stylet is cylindrical.

We have a series of both sexes from :

Santa Andrea, Tabasco, Mexico, off *Dicotyles labiatus*, May 1897, collected by Dr Buller.

Charuplaya, Bolivia, off *Speothos vinaticus*, June 1897, collected by P. O. Simons.

San Bernardino, Paraguay, off *Dasypsecta aguti*, May 1906, collected by Karl Fiebrig.

Minas Geraes, Brazil, no host, collected by A. Kennedy.

In the ♂♂ from Brazil and Paraguay the ninth abdominal sternite is longer, bearing fewer hairs proximally and more distally (Pl. VI, fig. 10) than in Mexican specimen.

#### (4) *Rhopalopsyllus cacicus spec nov.*

(Pl. III, fig. 13; VI, fig. 8.)

*Pulex australis* Rothschild (1904 a, p. 613, n. 8, partim, Perené).

Nearly related to *R. australis*, but differing in the following characters :

**Head.** The rostrum reaches to the apex of the forecoxa, the last

segment being somewhat shorter than the two preceding segments together. The second segment of the maxillary palpus is longer than in *R. australis*.

**Thorax.** The epimerum of the metathorax bears 6 or 7 bristles in two rows (3, 4; or 3, 3).

**Legs.** The mid- and hindtibiae bear a row of 4 or 5 bristles on the inner surface. The second midtarsal segment (Pl. III, fig. 13) is considerably longer than in *R. australis*. The bristles of the tarsi are shorter, the longest apical bristle of the first hindtarsal segment not reaching to the apex of the second segment, while the longest bristle of the second segment only reaches to the apex of the third. The longest apical bristle of the fourth hindtarsal segment hardly extends to the base of the third pair of bristles of the fifth segment.

**Modified segments.** ♂. The clasper bears near the insertion of the "finger" 4 bristles (3, 1), besides 2 small hairs. The ninth sternite (Pl. VI, fig. 8) is broader than in *R. australis* and bears many more hairs, one of them being long, while all the other ventral ones are short.

We have two ♂♂ from Perené, Peru, taken off *Tatusia novemcincta* by P. O. Simons.

(5) *Rhopalopsyllus lugubris spec. nov.*

(Pl. III, fig. 12; VI, fig. 9.)

Larger than *R. australis*, to which it is closely allied.

**Head.** The last segment of the rostrum is longer than the two preceding ones together. The second segment of the maxillary palpus, as in *R. cacicus*, is much longer than the fourth.

**Thorax.** The anterior row on the pronotum contains about 10 bristles on the two sides together. The epimerum of the mesothorax bears 4 long bristles, 2 of which are placed above the stigma, besides 2 or 3 short ones situated in front of the meral suture.

**Abdomen.** The basal abdominal sternite bears about 17 short bristles on the lateral surface, arranged in three irregular rows, while the second sternite has on each side 3 or 4 short bristles in front of the postmedian row.

**Legs.** The hind margin of the hindcoxa is much less rounded than in the allied species. The midfemur bears about 24 bristles on the outside and the hindfemur about 18, arranged in three irregular rows. The bristles on the outside of the mid- and hindtibiae are more numerous than in *R. australis* and *cacicus*, the hindtibia bearing about

18 bristles. The hairs on the outside of the tarsi are likewise more numerous. For the first and second midtarsal segments see Pl. III, fig. 12.

**Modified segments.** ♂. The eighth sternite is ventrally produced. The bristles at and near the dorsal edge of the clasper are more numerous than in the two preceding species, the ventral edge bearing 3 bristles distally to the insertion of the "finger," and one proximally to it. The ninth sternite has one small bristle at the apex and 3 long ones before it, the most proximal one of the three being the longest, there being also some more small bristles further proximad (Pl. VI, fig. 9).

**Length.** ♂ 2.6 mm.

One ♂ from Charuplaya, Bolivia, found by P. O. Simons on *Speothos vinaticus*, together with a ♂ of *R. australis*, January 1897.

In contradistinction to the five preceding species in which the fifth tarsal segment is long (Pl. III, fig. 10), in all the following species this segment is short (Pl. IV, fig. 4).

(6) *Rhopalopsyllus bohlsi* Wagn. (1901).

(Pl. IV, fig. 4; VII, fig. 7.)

*Pulex bohlsi* Wagner (1901, p. 21, n. 5, t. 1, fig. 6, ♀, Paraguay, 1 ♀).

*Rhopalopsyllus bohlsi* Baker (1905 a, pp. 130, 143).

**Head.** The frons bears an anterior row of 5 or 6 bristles which are very much thinner and shorter than the 3 eye-bristles. The first row of the occiput consists of 12 to 14 slender bristles, the second row of 16 and the third of 14 to 16, on the two sides together. In the ♂ there are, moreover, numerous short slender bristles in the dorsal groove of the occiput.

**Thorax.** The pronotum has two rows of bristles, the first consisting of about 16, the second of about 18 bristles, on the two sides together, not counting the small hairs which are situated in between the bristles of the second row. The numbers of bristles in the two rows of the mesonotum are about 22 and 16 respectively. The metanotum has three rows of about 16, 26, 16 bristles. On the pleura of the mesothorax there are 5 bristles, while the epimerum of the metathorax bears 10 or 11 in two rows (6 or 7, 4 or 5). The metanotum has 10 to 15 short apical spines.

**Abdomen.** The tergites bear each two rows of bristles, the anterior segments having a few additional bristles on the back. The numbers of bristles in these rows are:—i. 16 to 22, 12 or 13; ii. 22, 19 to 21; iii. 22 to 24, 19 or 20; iv. 18 to 21, 18 to 20; v. 15 or 16, 18 to 20; vi. 11 in ♂,

16 in ♀, 16 or 17; vii. 10 in ♂, and 13 or 14 in ♀, 12 to 14. On the sternites of the third to seventh segments there are in the ♂ 11, 12, 10, 10, and 10 bristles respectively, the numbers being in the ♀ 14, 12, 10, 11 to 13, and 15 on the two sides together, the seventh sternite having on each side 5 or 6 additional bristles in front of the row. There are also some small bristles before the row of long ones on the sternite of the third segment in the ♀. The basal sternite bears on each lateral surface in the ♂ about 5 hairs, in the ♀ 12 to 16, the segment having also along the ventral line 5 to 9 short hairs. The proximal tergites bear short apical spines, the numbers being in the ♂ 13, 8, 5, 4, 1 and in the ♀ 11 to 16, 5 or 6, 3 to 6, 3 or 4.

**Legs.** The forecoxa bears more than 50 bristles. The fore- and midfemora have a number of short bristles scattered over the outer surface, besides some longer subapical bristles. There is a row of about 8 bristles on the outer side of the hindfemur, and a row of 4 to 6 on the inside. The hindtibia bears 30 odd bristles on the outer side, inclusive of those placed at the anterior edge, the bristles being slightly more numerous in the ♂ than in the ♀. The longest apical bristle of the second hindtarsal segment does not reach to the middle of the fourth segment. There are 2 ventral apical bristles on the fifth segment, one being long the other short and stout, the fourth lateral pair, which stands close to these apical bristles, being also short, strong, and blunt. The fifth segment of the hindtarsi is about as long as the second midtarsal one (Pl. IV, fig. 4).

**Modified segments.** ♂. The eighth sternite bears on each side a row of 5 bristles, in front of which there are several smaller bristles. The triangular clasper (Pl. VII, fig. 7) bears a long bristle beneath the apex and about 9 medium-sized bristles along the upper edge, besides several small ones. At the ventral edge, not very far from the long subapical bristle, the clasper has a notch, the "finger" extending beyond this notch. The ninth sternite bears about a dozen bristles, one of which is much longer than the others. The manubrium of the clasper is somewhat widened at the apex.—♀. The eighth tergite bears 6 to 8 hairs above the stigma, of which the one nearest the apex is the longest, corresponding to the long subapical bristle of the seventh tergite. From the stigma downwards there is a row of 7 long and 14 short bristles, the segment bearing basally at some distance from that row a patch of about 5 short bristles. Along the ventral and apical edges there are 18 to 20 long and short bristles. The stylet is slightly bottle-shaped, bearing a small notch near the apex.

**Length.** ♂ 2.2 mm., ♀ 2.4 mm.

We have not seen the specimen described and figured by Wagner, but we believe we have correctly identified as *bohlsi* the undermentioned specimens received from Paraguay, Argentina and Ecuador.

One ♂ from Paraguay, off *Didelphys azarae*, Dec. 1901; W. Foster.

Two ♀ ♀ from Sapucay, Paraguay, off *Didelphys azarae*; W. Foster.

Two ♀ ♀ from Gran Chaco, Argentina, off *Didelphys azarae*, May 1900; Mr Pride (per J. Graham Kerr).

One ♀ from Ibarra, Ecuador, off *Nectomys saturatus*, May 1897; W. F. H. Rosenberg.

(7) *Rhopalopsyllus roberti* Rothsch. (1905).

*Pulex roberti* Rothschild (1905, p. 479, n. 1, t. 13, figs. 1, 2, São Paulo, off *Nectomys* and *Didelphys*).

Closely related to *R. bohlsi*; but *roberti* is larger than that species, and the bristles are much stouter, especially those situated on the head. There is an additional row of bristles dorsally in front of the two rows of the mesonotum and of the three rows of the metanotum. The long ventral apical bristle of the fifth foretarsal segment is stouter than in *R. bohlsi*. The apical bristles of the second hindtarsal segment are somewhat shorter than in that species. The eighth sternite of the ♂ bears on each side a curved row of 5 or 6 bristles, there being no additional bristles in front of this row. The bristles on the dorsal side of the clasper are fewer in number than in *R. bohlsi* and there is a long bristle at the point where the clasper joins the dorsal portion of the ninth segment. The bristles of the ninth sternite of the ♂ are all practically of the same length. In the ♀ the seventh abdominal sternite bears a single row of long bristles, there being no bristles in front of the row, and the eighth sternite has fewer bristles than in *bohlsi*.

We have both sexes from São Paulo, Brazil, off *Didelphys aurita*, found in November 1901 by A. Robert.

(8) *Rhopalopsyllus bernhardi* spec. nov.

(Pl. VII, fig. 6.)

Likewise very closely related to *R. bohlsi*. The first midtarsal segment is somewhat shorter. The clasper of the ♂ bears fewer bristles than in *R. bohlsi* and *roberti*, there being a large bristle (broken in our single specimen) where the clasper joins the dorsal portion of the ninth

segment. The "finger" of the clasper is one-fourth shorter than in *bohlsi* and *roberti*, and its bristles are placed closer together in the centre of the ventral margin. The manubrium is slenderer than in the allied species. The ninth sternite of the ♂ is very robust (Pl. VII, fig. 6). It is rounded at the apex, not bearing any bristles at the extreme tip, while the ventral bristles placed on the inner side close to the apex are very numerous. One of the bristles of the outside is longer than the others, as is the case in *R. bohlsi*.

With this ♂ a ♀ was sent which may belong to the same species, though it does not apparently differ from the ♀ of *R. bohlsi* except that the first midtarsal segment is slightly shorter, as is the case also in the ♂ of *bernhardi*.

One ♂ and one ♀ off a species of *Didelphys*; San Bernardino, Paraguay, May 1906, collected by Herr Karl Fiebrig.

(9) *Rhopalopsyllus platensis spec. nov.*

(Pl. VII, fig. 10.)

We have only one ♀ of this insect.

The rostrum reaches to the trochanter, being a little longer than in the ♀♀ of the allied species. The maxillary palpus is also longer, the segments measuring 20, 26, 14 and 26 respectively. The pleura of the mesothorax bears 6 bristles. The hindcoxa has only 2 bristles posteriorly at the apex. There are 24 bristles on each lateral surface of the basal abdominal sternite, besides the bristles which are situated at the ventral margin. The hindfemur bears on the outside a row of 9 or 10 bristles, and on the inside a row of 8. The bristles on the outside of the hindtibia are less numerous than in the allied species. The long bristle situated in the fourth incision of the hindtibia reaches far beyond the apex of the tibia. The longest apical bristle reaches beyond the tip of the first tarsal segment, the longest bristle of this segment extending beyond the apex of the second segment, while the corresponding bristle of the second segment extends close to the apex of the fourth segment. The bristles on the seventh abdominal sternite and the small lateral ones which stand on the eighth tergite (Pl. VII, fig. 10) basally to the lateral row are more numerous than in *R. bohlsi*.

One ♀ off *Ctenomys spec.* from La Plata, collected by Dr Spegazzini, and received from the late Dr C. Berg.

(10) *Rhopalopsyllus cavicola* Weyenb. (1881).

(Pl. IV, fig. 3.)

*Pulex cavicola* Weyenbergh (1881, p. 274, Argentina, off *Cavia leucopyga*); Rothschild (1906, p. 174, n. 3=*concitus*).

*Pulex concitus* Rothschild (1904 a, p. 615, n. 10, t. 10, figs. 38, 40, Bolivia, off *Herodon boliviensis*).

*Rhopalopsyllus concitus* Baker (1905 a, pp. 130, 143).

We only know the ♀ of this insect. The species is easily distinguished by the very long rostrum.

**Head.** The labial palpus consists of 5 segments, reaching beyond the apex of the trochanter. The first segment of the maxillary palpus is a little shorter than the second, while the third is one-third, or a little over one-third, the length of the fourth. The anterior frontal row of bristles contains 6 to 8 rather thin bristles, the fifth being the longest. There is one bristle beneath the eye, one behind the eye, and a row of 3 in front of it. The occiput bears three rows of rather slender bristles.

**Thorax.** The posterior row on the nota consists of 14 bristles, the anterior row of the pro- and mesonotum containing 12 bristles on the two sides together. On the metanotum there is a third-row of about 9 bristles in front of the other two, the second row consisting of about 15. The pleura of the mesonotum has 3 or 4 bristles, and the epimerum of the metathorax 7 or 8 in two rows (3 or 4, 4).

**Abdomen.** The tergites have all two rows of bristles. The first tergite, like the metanotum, bears a comb of short apical spines, there being also some spines on the following segments. The basal sternite has a lateral patch of about 15 to 20 small hairs, the segment bearing also some bristles ventrally near the apex. The next sternite has a row of 10 to 12 bristles on the two sides together, there being several short additional hairs in front of the row. On the sternite of the seventh segment there is a curved row of 6 or 7 bristles on each side, with some small ones in front. The apical bristle of the seventh tergite is longer than the first and second midtarsal segments together.

**Legs.** The forecoxa has less than 30 bristles. The midcoxa bears about 12 bristles along the anterior edge, apart from those which stand at the apical margin. The hindcoxa has 2 bristles posteriorly at the apex. The mid- and hindfemora bear one row of bristles on each side. The hindtibia has 12 bristles on the outer surface, besides 3 to 6 short ones, which are placed along the anterior margin. The apical bristles of the tibiae are long and rather thin, the longest one of the hindtibia

reaching to the apex of the first hindtarsal segment. The fifth segment is short, being in the foretarsus (Pl. IV, fig. 3) half as long again as it is broad. The measurements of the mid- and hindtarsi are as follows:

Segment	First	Second	Third	Fourth	Fifth
Midtarsus :	15	19	11	7	22
Hindtarsus :	38	26	17	9	24

**Modified segments.** ♀. The apical margin of the eighth tergite is rounded, bearing on the outside a regular row of 7 to 9 long bristles and on the inside about a dozen shorter ones. There is on the outside a row of 3 or 4 long and 4 to 7 short bristles running from the stigma downwards, while there are usually 4 bristles above the stigma, the apical one being the largest. The stylet is short, being about half as long again as it is broad.

**Length.** ♀ 2.4 mm.; an exceptionally small specimen from Bolivia 1.7 mm.

We have 6 ♀♀ as follows: two cotypes of *cavicola* from the collection of the late Professor Weyenbergh; and four specimens from Sucre, Bolivia, off *Herodon boliviensis*, 6th September, 1901, collected by P. O. Simons.

(11) *Rhopalopsyllus litus spec. nov.*

This is the only known species of *Rhopalopsyllus* in which the occiput bears only two rows of bristles.

**Head.** The bristles are slender. The anterior row of the frons consists of 5 bristles. The bristles situated beneath and behind the eye are apparently small (they are broken off in our two specimens). The anterior row of bristles of the occiput is absent. The second row contains only 2 fairly long bristles and 1 or 2 small hairs. The rostrum reaches the apex of the forecoxa, the labial palpus consisting of five segments. The first segment of the maxillary palpus is longer than the third, the second being a little shorter than the fourth.

**Thorax.** The posterior row of bristles on the nota consists of 14 bristles, the antemedian row containing on the pronotum about 12 small bristles and on the meso- and metanotum about 18, the metanotum bearing an additional row of 8 or 9 short bristles. There are 5 long bristles on the pleura of the mesothorax and 8 to 11 bristles in two rows on the epimerum of the metathorax (4 to 6, 4 or 5).



**Abdomen.** The first tergite has, like the metanotum, the usual comb of short apical spines, a few such spines occurring also on the next two or three tergites. There are two rows of bristles on the tergites, the first row containing in our two specimens 12 and 13, 16 and 22, 14 and 20, 12 and 19, 11 and 19, 10 and 11, 8 and 10, bristles respectively, the second row consisting of 10 and 11 bristles on the first segment, 16 and 17 on the second to fifth, 13 and 11 on the sixth, and 13 and 14 on the seventh respectively. The apical bristle of the seventh tergite is as long as the first and second midtarsal segments together. The basal sternite bears a large patch of small hairs on the side. The next sternite has a row of 14 or 18 bristles on the two sides together, the upper bristles being short and there being also several short bristles in front of the row. The sternites of segments 4, 5 and 6 bear each a row of 8 or 12 or 14 bristles, the seventh sternite having a row of about 12 and ventrally in front of the row 4 or 6 more. As in *cavicola* the edge of the pygidial plate is only slightly raised anteriorly, projecting much less than in *R. australis*.

**Legs.** The forecoxa bears about 40 bristles. The hindcoxa has 3 bristles posteriorly at the apex. The mid- and hindfemora bear a row of bristles both on their inner and outer sides, there being a number of additional bristles on the lateral outer surface of the midfemur and 1 to 3 subapical lateral bristles on the outside of the hindfemur. The hindtibia has about 10 lateral bristles on the outside and about 10 shorter ones along the anterior (= ventral) side. The longest apical bristle of the hindtibia does not reach to the apex of the first tarsal segment, while the longest apical bristle of the second hindtarsal segment extends to the base of the fourth segment. The third segment of the foretarsus and the fourth of the hindtarsus are much longer than they are broad, the third midtarsal segment being about twice as long as it is broad. The fifth tarsal segment is small, being in the foretarsus twice as long as it is broad. The measurements of the mid- and hindtarsi are in the larger and more hairy specimens (the type of name) 20, 29, 15, 9, 24, and 57, 41, 22, 12, 26, respectively; in the smaller specimens the segments measure in the midtarsus 17, 22, 12, 8, 21, and in the hindtarsus 46, 30, 17, 9, 22.

**Modified segments.** ♀. The eighth tergite bears 6 or 7 bristles above the stigma in the larger specimen, and 4 in the smaller one, the apical bristle being far stouter than the others. From the stigma downwards there is, in the larger individual, a row of 6 or 7 large bristles and 6 to 10 small ones, while there are about 10 bristles along the

ventral and apical margins on the outer side and about as many shorter ones on the inner side. The number of bristles is slightly smaller in the second specimen. Proximally to the lateral row there are some short hairs in both examples. The stylet is about twice as long as it is broad.

**Length.** ♀ 2.9 mm. and 2.3 mm.

We have two ♀♀ without host and locality, being most probably South American.

(12) *Rhopalopsyllus klagesi* Rothsch. (1904).

(Pl. VII, figs. 8 and 9.)

*Pulex klagesi* Rothschild (1904 a, p. 620, n. 14, t. 9, fig. 28, t. 10, figs. 34, 39, Caura R., Venezuela, off *Prochimys*).

*Rhopalopsyllus klagesi* Baker (1905 a, pp. 130, 144).

A small species with the spines of the head strongly developed.

**Head.** The rostrum does not quite reach to the apex of the fore-coxa, the labial palpus consisting of five segments. The first segment of the maxillary palpus is a little longer than the third, and the second longer than the fourth. There are two rows of bristles on the frons, besides a large bristle situated beneath the eye and another behind the eye. The occiput bears three rows of bristles.

**Thorax.** The pro- and mesonotum have two rows of bristles each, the first consisting in the ♀ of about 12, the second of 14 bristles. On the metanotum there are three rows of bristles (in ♀ 5 to 10, 16, 12). The ♂ has a few bristles less in these rows than the ♀. The pleura of the mesothorax has 5 strong bristles. The epimerum of the metathorax bears 7 to 9 bristles in two rows (3 to 5, 3 or 4), the first bristle of the anterior row being placed near the upper corner of the sclerite.

**Abdomen.** In the ♂ the anterior row of bristles consists of about 10 bristles on the first tergite, 6 on the second, and 1 or 2 on the third to seventh tergites, the posterior row containing 8 bristles on the first tergite, 14 on the second, and 13 or 12 on the others. In the ♀ the number of bristles in the anterior row of the four proximal tergites are about 12, 14, 12, 10, and on the fifth, sixth and seventh tergites 8, the posterior row consisting of 9 or 10 bristles on the first tergite, 14 on the next three, and 12 or 13 on the fifth to seventh. The first tergite bears an apical comb of short spines like the metanotum, the second to fifth, sometimes even sixth, having only one or more such spines. The basal sternite bears in the ♂ 1 to 4 minute

hairs on the side, and 8 to 10 in the ♀. On the sternites of segments three to six there is a row of 4 to 6 bristles on both sides together, the ♀ having 6 to 9 bristles, there being some additional hairs in front of the row on the sternite of the third segment. The sternite of the seventh segment has one or two bristles less than the preceding segment. The edge of the pygidial plate is very slightly raised anteriorly.

**Legs.** The forecoxa bears less than 30 bristles. The midcoxa has about 8 to 10 bristles at and near the anterior edge, there being no bristles on the inner surface. The hindcoxa has 3 bristles posteriorly near the apex and likewise no bristles on the inside near the anterior margin. The hindfemur has a row of 5 to 8 bristles outside and only 3 widely separated bristles inside. The hindtibia has 13 lateral bristles besides a number of small hairs which are placed at the anterior edge. The longest (ventral) apical bristle of the hindtibia extends in both sexes beyond the apex of the first hindtarsal segment, the longest bristle of this segment reaching in the ♂ beyond the apex of the second segment, but is somewhat shorter in the ♀. The fifth tarsal segment is short. In the foretarsus it is very broad, being about half as long again as it is broad, while in the hindtarsus it is very slender. The lateral bristles of this segment are rather thin and long, the third bristle extending close to the apex of the segment. The measurements of the mid- and hindtarsi are:

Segment		First	Second	Third	Fourth	Fifth
Midtarsus:	♂	18	24	15	9	21
	♀	18	31	19	9	25
Hindtarsus:	♂	40	34	18	10	24
	♀	52	42	24	12	29

**Modified segments.** ♂. The eighth sternite bears about 6 bristles on the two sides together. The clasper is acuminate (Pl. VII, fig. 8). It bears a number of hairs at the upper margin, 2 bristles beneath the apex and 3 thin hairs (2, 1) at the ventral margin near the base of the "finger" (F). The two posterior thin hairs were originally erroneously described as being situated on the finger. The manubrium (M) is slender. The finger reaches to the tip of the clasper. The ventral portion of the ninth sternite (ix. st.) is short and slender.—♀. The eighth tergite is very strongly produced apically (Pl. VII, fig. 9). There are 3, 4 or 5 hairs above the stigma, one of them being thick. A row of 2 large and several small bristles extends from the

stigma downwards, 1 or 2 more large bristles being placed further ventrally. The ventral and apical edges also bear an irregular row of long and short bristles as shown in the figure. The stylet is cylindrical.

**Length.** ♂ 1.4 mm., ♀ 2.2 mm.

We have both sexes from Maripa, Caura River, Orinoco, off *Prochimys spec.*, May 1903, collected by S. M. Klages.

6. Genus: **Parapsyllus** Enderl. (1903).

*Parapsyllus* Enderlein (1903, p. 260, name-type: *longicornis* Enderl.); Baker (1905 a, p. 131).

♂ ♀. Nearest to *Rhopalopsyllus*, from which it differs especially in the antenna.

**Head.** The genal process short, obtuse, bearing a number of bristles (Pl. II, fig. 12). The antennal groove large, open behind, extending on to the prosternite. The club of the antenna long, resembling that of *Ceratophyllus* Curt., being acuminate in the ♂, with the last segment ovate and the segmental incisions distinct all round the club. The proximal segments of the club symmetrical, not being semi-detached and not sloping backwards as in *Rhopalopsyllus*. The short hairs situated at the upper edge of the antennal groove thinner than in *Rhopalopsyllus*, being in the ♀ few in number and placed widely apart. The labial palpus has 4 or 5 segments.

**Thorax.** The thoracical tergites bear two rows of bristles, there being usually some additional bristles on the meso- and metanotum. The metanotum has no apical spines. The episternum of the metathorax is smaller than in *Rhopalopsyllus*.

**Legs.** No comb of spines on the hindcoxa.

**Abdomen.** The stigmata are smaller than in *Rhopalopsyllus*.

**Modified segments.** These are of the same type as in *Rhopalopsyllus*; but in one species (*simonsi*) the clasper bears an additional process.

The species, as far as they are known, are so different from each other that they possibly represent four genera. These species are, however, more closely allied to one another than to the other non-combed eyed *Pulicidae* and, moreover, have some conspicuous characters in common, that we consider it unnecessary to propose several new genera for their reception. They will, therefore, be placed under Enderlein's generic term, till the discovery of a larger number of species renders it necessary to divide the genus.

The species are South American and Antarctic, one species (*longicornis*) going with its host (Penguin) northward to Australia.

### Key to the species.

- a. Labial palpus consisting of 4 segments .....Species No. 4
- Labial palpus consisting of 5 segments .....b
- b. Proximal segments of abdomen with apical spines .....Species No. 3
- Proximal segments of abdomen without apical spines .....c
- c. Mesonotum without thin bristle-like subapical spines .....Species No. 1
- Mesonotum with thin bristle-like subapical spines .....Species No. 2

### (1) *Parapsyllus longicornis* Enderl. (1901).

(Pl. II, fig. 12; IV, fig. 5; VII, fig. 3.)

*Pulex longicornis* (1901, p. 553, t. 34, figs. 8, 9, 12, S. Paul Is., off *Eudyptes* "chrysocome").

*Parapsyllus longicornis* Enderlein (1903, p. 261, fig. 2, t. 39, figs. 13, 14, 16, 17, 19, 21); Baker (1905 a, p. 144).

**Head.** The frontal tubercle is large (Pl. II, fig. 12), being situated close above the frontal oral corner of the head. There is a row of bristles beneath the eye along the genal edge. The antennal groove is very large in the ♂, there being in both sexes a distinct internal incrustation of the skeleton from the base of the antennal groove upwards. The second antennal segment bears a row of long bristles along the apical edge, the bristles being especially long in the ♀, some of them reaching to the apex of the club in this sex. The rostrum extends to the trochanter, the labial palpi consisting of five segments.

**Thorax.** The mesonotum has no subapical spines. The epimerum of the metathorax bears a row of 3 or 4 bristles running from the stigma downwards, and 1 to 3 additional bristles representing an anterior row. The episternum of the metathorax is longer in a vertical direction than horizontally. There is no external suture between this episternum and the metanotum.

**Abdomen.** There are no apical spines on the tergites. The tergites bear each two rows of bristles, the anterior row being more or less incomplete on the posterior segments of the ♂, while the ♀ has some additional bristles on the sixth and seventh tergites. The basal sternite bears a number of small hairs on the side, the other sternites have a postmedian row of bristles and in front of it several small hairs, which are more numerous in the ♀ than in the ♂.

**Legs.** The mid- and hindcoxae are long and narrow. The hind-femur bears a row of about 8 bristles on the inner side, there being on the outer side in the ♂ 1 or 2 bristles, and in the ♀ a row of about 5, besides 3 or 4 present in both sexes and more dorsal in position. The hindtibia bears on the outside two lateral rows of bristles, one row being placed near the dorsal bristles and the other near the ventral (= anterior) edge, this edge bearing some additional bristles. The first and second midtarsal segments are equal in length, or the first is but a very little longer than the second. The first, second and third hindtarsal segments are rather strongly dilated towards the apex, the fourth segment being more than twice as long as it is broad. The longest apical bristle of the second segment does not quite reach to the apex of the fourth segment. The third pair of lateral bristles of the fifth segment (Pl. IV, fig. 5) are situated at the lateral edge of the segment like the other lateral bristles. There is only one small hair on the ventral surface of this segment.

**Modified segments.** ♂. The apex of the large eighth sternite is rounded, the segment bearing a postmedian row of bristles and a number of smaller bristles further basad. The clasper (Pl. VII, fig. 3, Cl) is large, quadrangular, the upper apical corner being produced, while the ventral apical corner is completely rounded off. There is a regular row of bristles along the apical edge, the dorsal edge also bearing bristles. The finger (F) is slender, subcylindrical, and slightly acuminate. The manubrium (M) also is slender, widening apically, and forming a small hook. The ventral portion of the ninth sternite (ix. st.) is canoe-shaped, the apex bearing about 10 bristles.—♀. The apical edge of the sixth sternite is straight. The seventh sternite bears a deep narrow sinus, the lobe above this sinus being small and the one below it large. On the eighth tergite there are 6 or more small bristles above the stigma, a row of 4 or 5 bristles on the side, and several small bristles proximally to this row, as well as between this row and the ventral margin. Along the apical edge of the eighth tergite there are about 12 bristles on the outside and more than 12 on the inside. The stylet is long, being subcylindrical.

**Length.** ♂ 2.1 mm., ♀ 3 mm.

This species was discovered on the Island of St Paul, where it was found on *Eudyptes chrysolophus* Reichenow.

We have 2 ♂♂ and 2 ♀♀ taken off *Endyptula minor* on Bird Island, near Perth, in West Australia (J. Burton Cleland).

These specimens do not agree exactly with Enderlein's figures, and may be a closely allied new species. Some of the figures, however, are

apparently inaccurate in detail, so we have identified our specimens as *longicornis*, in spite of all discrepancies. The figure given by Enderlein of the male genitalia is misleading. This figure also shows 2 antepygidial bristles, while our specimens have only one such bristle, like all members of the genera *Parapsyllus* and *Rhopalopsyllus*. In Enderlein's figure of the ♀, however, only one such bristle is drawn on each side. We may therefore assume that the 2 bristles in the figure of the ♂ are an oversight. In the figure of the ♀ the anal segment bearing the stylet is left out altogether, and the small hairs on the mid- and hind-coxae, on the abdominal sternites and on the eighth tergite are too numerous.

(2) *Parapsyllus simonsi* Rothsch. (1904).

(Pl. IV, fig. 1.)

*Pulex simonsi* Rothschild (1904 a, p. 616, t. 9, fig. 30, t. 10, fig. 37, Bolivia, off *Neotodon simonsi*).

*Rhopalopsyllus simonsi* Baker (1905 a, pp. 130, 144).

**Head.** The frontal tubercle is situated close to the frontal oral corner. There is a row of bristles beneath the eye along the edge of the genal process. The antennal groove of the ♂ extends close to the crown of the head, there being a distinct internal incrossation from the base of the groove upwards; in the ♀ this incrossation is vestigial. The rostrum reaches to the apex of the forecoxa, the labial palpus consisting of five segments.

**Thorax.** The mesonotum has a subapical series of thin long spines. The metathoracic epimerum bears a row of 3 (♂) or 4 (♀) bristles running from the stigma downwards.

**Abdomen.** There are no apical spines on any of the tergites. The basal tergite has a large patch of small hairs on the side. The tergites bear two rows of bristles in both sexes, the anterior row, which is more or less incomplete on all the segments, being represented by only a few bristles on the posterior tergites of the ♂.

**Legs.** The mid- and hindfemora bear a row of 7 or 8 bristles on the outside and a row of about 12 on the inner side. The dorsal and apical bristles of the hindtibia (Pl. IV, fig. 1) are extremely long and thin in the ♂, the long bristle of the fifth dorsal pair being longer than the tibia. The first midtarsal segment is a little longer than the second. The first hindtarsal segment of the ♂ is nearly as long as the second, third and fourth together. The first, second and third hindtarsal

segments have each 1 or 2 very long thin apical bristles in the ♂ (the tarsi of our single ♀ are broken). The third pair of bristles of the fifth tarsal segment is placed more towards the centre of the segment than is usual, the sole of this segment having a number of small hairs.

**Modified segments.** ♂. The eighth sternite, which is large, bears on each side close to the ventral apical corner three long bristles, besides a number of other bristles placed on the lateral surface. The clasper is very large, narrowing into a large curved manubrium. The dorsal apical corner of the clasper is somewhat prolonged upwards, while the ventral margin bears a long slender process furnished with three long bristles at the apex. Along the posterior edge of the clasper there is a row of long bristles. The external horizontal portion of the ninth sternite is also large, the lower apical angle being acuminate. This segment bears three patches of bristles.—♀. The seventh sternite is broadly emarginate, there being no lobe above this shallow sinus, while there is a broad lobe beneath it. The sixth sternite also bears a small sinus. The eighth tergite has a few bristles above the stigma, a row of about 6 long ones running from the stigma downwards, and an apical row of about 8, besides some short bristles situated near the longer apical ones. The stylet is cylindrical.

**Length.** ♂ 2.1 mm., ♀ 4 mm.<sup>1</sup>

We have 2 ♂♂ and 1 ♀ from Challapata, Bolivia, off *Octodontomys* (= *Neotodon*) *simonsi*, 11th October, 1901; and another ♂ from Potosi, Bolivia, off *Akodon albiventer*, 26th September, 1901; P. O. Simons.

### (3) *Parapsyllus cocyti* Rothsch. (1904).

(Pl. II, fig. 2; VI, fig. 5.)

*Pulex cocyti* Rothschild (1904 a, p. 617, n. 12, t. 9, fig. 26, t. 10, fig. 31, Chile, off Burrowing Rat).

*Rhopalopsyllus cocyti* Baker (1905 a, pp. 130, 143).

**Head.** The rostrum reaches to the apex of the forecoxa, the labial palpus consisting of five segments. There are a number of small hairs beneath the eye, one of them being situated at the tip of the genal process. The frontal tubercle is placed near to the frontal oral angle. There is no incrassation from the antennal groove upwards in either sex.

**Thorax.** The tergites bear each two rows of bristles, the anterior row not extending so far down as the posterior one. The mesonotum,

<sup>1</sup> In this specimen the segments are extended.



moreover, has a row of thin subapical spines (Pl. II, fig. 2); both the meso- and metanotum have some additional short bristles dorsally in front of the rows.

**Abdomen.** The tergites have two rows of bristles: the anterior row, however, is incomplete, especially in the ♂, being in this sex represented by only a very few bristles on the central and posterior segments. The posterior row contains on the central segments 14 or 16 bristles on the two sides together. The basal sternite has no bristles on the side. The sternites of segments 3 to 6 bear a row of 6 to 8 bristles on the two sides together, there being no additional bristles in front of this row, the sternite of the seventh segment bearing in the ♀ 10 to 12 bristles.

**Legs.** The posterior edge of the hindcoxa is only slightly rounded. The mid- and hindfemora bear one subapical ventral bristle on the outside and 2 to 4 lateral ones near the base, there being on the inside one row of bristles, containing on the hindfemur about 6 bristles in the ♂ and 9 in the ♀. The tibiae bear one row of lateral bristles. The first midtarsal segment is shorter than the second. The fourth hindtarsal segment is one-third longer than it is broad.

**Modified segments.** ♂. The clasper (Pl. VI, fig. 5, Cl) is almost square, the upper angle being slightly acuminate, and the lower angle rounded off. There is a row of bristles along the distal and dorsal edges as shown in the figure (Pl. VI, fig. 5). The finger (F) is small, elongate-conical, bearing a row of bristles at the distal edge. The ninth sternite is slightly curved, the obtuse tip being somewhat dilated.—♀. The eighth tergite bears a row of 8 or 9 bristles along the apical and ventral edges, there being between this row and the stigma 6 or 7 more bristles on the side; on the inner surface of the segment there is a dense patch of bristles near the apex. The stylet is cylindrical, being twice as long as it is broad.

**Length.** ♂ 1.45 mm., ♀ 2.1 mm.

A small series of both sexes from the Coast Hills, Chile, off Burrowing Rat; also from Valparaiso, no host being given; J. A. Wolffsohn.

(4) *Parapsyllus corfidii* Rothsch. (1904).

(Pl. VI, fig. 6.)

*Pulex corfidii* Rothschild (1904 a, p. 619, n. 13, t. 9, fig. 27, t. 10, fig. 33, Valparaiso, off *Octodon degus*).

*Rhopalopsyllus corfidii* Baker (1905 a, pp. 130, 144).

**Head.** The frons is much more strongly rounded than in the other

three species of *Parapsyllus*, the frontal tubercle, moreover, being placed farther away from the frontal oral corner than is the case in the other species. The bristles, too, at the genal edge are more numerous. The rostrum does not reach to the apex of the forecoxa, the labial palpus consisting of only four segments, of which the last is much the longest. The internal incrassation of the skeleton from the antennal groove upwards is vestigial in the ♂, and quite absent from the ♀.

**Thorax.** The thoracical tergites have each two rows of bristles, the mesonotum bearing an additional row, besides a row of subapical long slender spines. The metathoracic episternum is longest in a vertical direction. The epimerum of the metathorax has two rows of bristles (5 to 7, 4 or 5).

**Abdomen.** The proximal tergites bear short apical spines, the comb consisting of 15 or 16 spines on the first tergite. The tergites have each two rows of bristles, there being some additional small bristles on the seventh tergite in the ♀. The bristles of the rows are very numerous, there being in the posterior row of the central segments some 30 bristles in the ♀ and about 24 in the ♂. The sternites have a row of about 16 bristles on the two sides together, with additional bristles in front, the basal sternite bearing a row of small bristles on the side with some additional hairs before it.

**Legs.** The bristles situated anteriorly on the outer surface of the broad and rounded hindcoxa are very numerous. All the femora and tibiae bear also very numerous bristles on the outside. These bristles are more or less arranged in rows, being more numerous in the ♂ than in the ♀, the tarsi also bearing numerous bristles on the outer surface. There is a row of bristles on the inner side of the mid- and hindcoxae. The first midtarsal segment is a little longer than the second. The fourth hindtarsal segment is nearly twice as long as it is broad.

**Modified segments.** ♂. The clasper is triangular, with the ventral margin strongly rounded (Pl. VI, fig. 6). The manubrium (M) is very broad, becoming gradually narrower and curving upwards apically. There are 4 long and several short bristles at the ventral and distal edges of the clasper (Cl). The finger (F) is small and conical, being slightly curved and bearing a number of small bristles. The ventral portion of the ninth sternite (ix. st.) is almost straight, being slightly rounded ventrally and bearing a number of bristles on the distal half.—♀. The apical edge of the seventh sternite slopes upwards. The eighth tergite bears two rows of small bristles above the stigma. There is a row of about 10 bristles running from the stigma downwards and

proximally to this row another composed of smaller bristles. Along the apical edge of the eighth tergite there is a row of about a dozen bristles on the outside, the inside bearing two rows of smaller but rather stout bristles.

**Length.** ♂ 1.4 mm., ♀ 2 mm.

1 ♂ and 3 ♀ from Valparaiso, Chile, off *Octodon degus* and *Abrocoma bennettii*, collected by J. A. Wolffsohn.

#### 7. Genus: *Coptopsylla* gen. nov.

**Head.** Frons truncate (Pl. II, fig. 4); no distinct tubercle above the truncate part. Antennal groove open, the genal process being very short; no distinct internal incrassation from the base of the groove to the vertex (♀). Club of antenna long, segmented all round as in *Parapsyllus*. Labial palpus very long, consisting of 5 segments. No bristles beneath the eye at the edge of the genal process.

**Thorax.** One row of bristles on the tergites; the mesonotum bearing some long thin spines before the apex. Epimerum of mesothorax nearly horizontal, covering the stigma. Sternum of metathorax long in a dorso-ventral direction.

**Abdomen.** Tergites with one row of bristles, except the first, which bears two; seventh tergite with a long and a short bristle on a tubercle placed at the apical edge, the latter being excised where the tubercle is placed; the edge between the tubercles of the two sides produced backwards. There are two receptacula seminis as in *Hystriopsylla* and *Macropsylla*.

**Legs.** Internal rod-like incrassation of midcoxa forked below middle. Hindcoxa without comb, excised posteriorly before apex, the angle distinct. First fore- and midtarsal segment shorter than second. Fifth tarsal segment with 5 lateral bristles.

**Modified segments.** ♀. Eighth abdominal tergite bearing some hairs above the stigma. Stylet with an apical bristle and a short one before apex situated in a notch. The ♂ not known to us.

Type of genus: *Pulex lamellifer* Wagner (1895). The genus contains only one species. It is nearly related to *Parapsyllus*, but can easily be recognised by the truncate frons, the 5 lateral bristles on the fifth tarsal segment, and the double receptaculum seminis.

(1) *Coptopsylla lamellifer* Wagn. (1895).

(Pl. II, fig. 4.)

*Pulex lamellifer* Wagner (1895, p. 504, fig. 1, ♂, Transcaspia, from nest of a rodent);  
Wagner (1898, p. 576); Baker (1904, p. 437).

The rostrum of this insect reaches beyond the middle of the forefemur.

Bolschoj Balchan, Transcaspia, in a rodent's nest found in the ground in May. Both sexes were obtained.

We have one ♀ of this species received from Dr J. Wagner.

8. Genus: *Goniopsyllus* Baker (1905).

*Goniopsyllus* Baker (1905 a, pp. 128, 140, type of name: *Pulex kerguelensis* Tasch.).

**Head.** Frons without tubercle but obtusely angulate. Eye placed very low down. A row of bristles from upper oral corner towards base of antenna and several more bristles further back. Antennal groove open behind, extended to vertex in ♂. Club of antenna long, segmented all round, being similar to the club of *Cerutophyllus*. A few small hairs above the antennal groove. Labial palpus consisting of five segments.

**Thorax.** Pronotum with two rows of bristles and some additional hairs before these rows. Meso- and metanotum densely hairy, the hairs being thin and short, except those of the postmedian row. No subapical spines on mesonotum. Epimerum of mesothorax covering the stigma. Episternum of metathorax small.

**Abdomen.** Densely hairy. Stigmata lanceolate. Proximal tergites with some small apical teeth; seventh tergite with 2 long apical bristles on each side, placed on a tubercle at the edge. Sternites also densely hairy, except basal one. Sensory plate (pygidium) strongly convex, projecting backwards.

**Legs.** Internal rod-like incrassation of midcoxa dividing about centre. No comb on hindcoxa. First midtarsal segment longer than second; fifth tarsal segment narrow, the bristles small, 5 on each side and 3 or 4 ventrally near the apex (Pl. IV, fig. 2).

**Modified segments.** ♂. Clasper with one movable process (the finger), which is long; ninth sternite boomerang-shaped, the internal, vertical arm pointed, the point being directed frontad, the horizontal arm spinose at apex, suggesting this sternite of *Hystrihopsylla*.—

♀. Seventh sternite sinuate. Anal segment very long, as it is also in the ♂. Stylet cylindrical, about twice as long as it is broad.

Most nearly related to *Hystrihopsylla* Tasch. (1880) and *Macropsylla* Rothschild (1905). The female possibly has two receptacula seminis as in the genera mentioned. The two ♀ specimens, however, contained in the British Museum are not well enough preserved for deciding the question.

The genus so far contains only one species.

(1) *Goniopsyllus kerguelensis* Tasch. (1880).

(Pl. IV, fig. 2; VII, fig. 11.)

*Pulex spec.* Eaton (1875, p. 2, "A *Pulex* is parasitic on *Haladroma*, and one (possibly the same) on *Diomedea fuliginosa*").

*Pulex kerguelensis* Taschenberg (1880 a, p. 67, n. 7, t. 2, fig. 12, ♀, Kerguelen); *id.* (1880 a, p. 123, host: *Pelecanoides urinatrix*); *id.* (1880 b, p. 169); Baker (1895, p. 65); Rothschild (1895, p. 66, Antipodes).

*Pulex verguelensis* Wagner (1898, p. 576).

The abdominal tergites are emarginate dorsally.

There are in the British Museum one ♂ and two ♀♀ of the original four specimens obtained by the Rev. A. E. Eaton off *Pelecanoides urinatrix* on Kerguelen Island during the Transit-of-Venus Expedition<sup>1</sup>. The flea off *Diomedea* mentioned by Eaton, *l.c.*, is apparently not contained in the British Museum's collection.

We have one ♂ from Antipodes Island, off *Platycercus unicolor*, collected by Mr M. Dannefaerd.

9. Genus: *Lycopsylla* Rothschild (1904).

*Lycopsylla* Rothschild (1904 a, p. 602, name-type: *L. novus*); Baker (1905, p. 127, a new family proposed for its reception).

**Head.** Frons with a tooth-like tubercle about half-way between the oral angle and the occiput. The lower oral angle produced downwards into a slightly curved triangular lobe. Genal process pointed, closing the antennal groove. An internal incrassation from the base of the antennal groove upwards. Club of antenna similar to that of *Parapsyllus*. Labial palpus consisting of four segments, segmentation well marked all round, tip of last segment symmetrical, with 3 hairs on each side, as in *Rhopalopsyllus* and *Goniopsyllus*.

<sup>1</sup> An account of this Expedition is published in *Philos. Trans. Roy. Soc.*, vol. CLXVIII. (1879) (extra volume), the four examples of this flea being recorded on p. 118.

**Thorax.** Mesonotum with a row of thin subapical spines.

**Abdomen.** One row of bristles on the tergites, there being an anterior additional row on the first tergite. Seventh tergite without antepygidial bristles in both sexes. Sensory plate not convex in side-view. ♀ without stylet.

**Legs.** Internal rod of midcoxa forking about centre. Hindcoxa without comb of short spines on the inside. Bristles of tibiae and tarsi stout. First midtarsal segment shorter than the second. Fifth tarsal segment long, with 6 (rarely 5) lateral bristles, besides the subapical hair. Claw long and slender, non-dentate, with the basal projection vestigial.

**Modified segments.** Of a similar type as in *Rhopalopsyllus* and *Parapsyllus*. The bristles on the anal segment of the ♀ very numerous.

Only one species is known of this Australian genus.

(1) *Lycopsylla novus* Rothschild. (1904).

*Lycopsylla novus* Rothschild (1904 a, p. 602, n. 1, t. 7, figs. 1—4); Baker (1905 a, p. 139).

We have both sexes from Hampden, New South Wales, off *Phascolomys mitchelli*, collected December 17, 1899, by Dr J. P. Hill.

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## EXPLANATION OF PLATES.

## PLATE I.

Fig. 1. *Loemopsylla cheopis* ♂.

## PLATE II.

Fig. 1. Head and thorax of *Pariodontis riggenbachi* ♂.

pron = pronotum ; meson = mesonotum ; metan = metanotum ; sti = stigma ;  
st = sternum ; est = episternum ; em = epimerum.

Fig. 2. Lateral view of mesothorax of *Parapsyllus cocyti*.

Fig. 8. Head and prothorax of *Rhopalopsyllus cleophontis*.

Fig. 4. Head of *Coptopsylla lamellifer* ♀.

Fig. 5. Head of *Loemopsylla chephrenis*.

Fig. 6. Hindfemur of *Loemopsylla erilli* ♂.

Fig. 7. The same of *Loemopsylla chephrenis* ♂.

Fig. 8. Apex of labial palpus of *Loemopsylla cheopis*.

Fig. 9. The same of *Rhopalopsyllus cleophontis*.

Fig. 10. Apex of the rostrum (=the two labial palpi) of *Loemopsylla divergens*.

Fig. 11. The same of *Loemopsylla creusae*.

Fig. 12. Head of *Parapsyllus longicornis* ♂ (drawn from a mounted specimen).

Fig. 13. Head and thorax of *Moeopsylla ejoestedti*.

Fig. 14. Hindcoxa of *Loemopsylla regis* ♂.

Fig. 15. The same of *Loemopsylla erilli* ♂.

Fig. 16. The same of *Loemopsylla isidis* ♂.

Fig. 17. Frontal view of head of *Pariodontis riggenbachi*.

## PLATE III.

Fig. 1. First hindtarsal segment of *Loemopsylla gerbilli* ♀.

Fig. 2. The same of *Loemopsylla mycerini* ♀.

Fig. 3. Apex of the hindtibia and the first and second hindtarsal segments of *Loemopsylla nesiotis* ♀.

Fig. 4. Midtarsus and apex of the midtibia of *Loemopsylla pallidus*.

Fig. 5. The first to fourth midtarsal segments of *Loemopsylla chersinus* ♂.

Fig. 6. The same of *Loemopsylla nubicus* ♂.

Fig. 7. Hindtarsus of *Loemopsylla cleopatrae* ♀, 2 to 5 segments.

Fig. 8. Midtarsus and apex of the midtibia of *Loemopsylla somalicus* ♂.

Fig. 9. Hindtibia and apex of the hindfemur of *Loemopsylla longispinus* ♀, the longest apical bristles being broken at the tip.

Fig. 10. Fifth hindtarsal segment of *Rhopalopsyllus cleophontis* ♀, ventral aspect.

Fig. 11. First and second midtarsal segments of *Rhopalopsyllus australis* ♂.

Fig. 12. The same of *Rhopalopsyllus lugubris* ♂.

Fig. 13. The same of *Rhopalopsyllus cacticus* ♂.

## PLATE IV.

Fig. 1. Hindtibia of *Parapsyllus simonsi* ♂.

Fig. 2. Fifth midtarsal segment of *Goniopsyllus kerguelensis* ♂.

Fig. 3. Fifth foretarsal segment of *Rhopalopsyllus cavicola* ♀.

Fig. 4. Fifth hindtarsal segment of *Rhopalopsyllus bohlsi* ♀.

Fig. 5. The same of *Parapsyllus longicornis* ♀.

Fig. 6. Genitalia of *Loemopsylla nubicus* ♂.

Cl.=clasper; Pen.=penis; Pen.-Pl.=penis-plate; P<sup>1</sup> and P<sup>2</sup> processes of the clasper; IX. st.=ninth sternite; IX. t.=ninth tergite; X. t.=tenth tergite; X. st.=tenth sternite; M=manubrium.

Fig. 7. The same of *Loemopsylla cleopatrae* ♂.

Fig. 8. The same of *Loemopsylla cheopis* ♂.

Fig. 9. The same of *Loemopsylla pallidus* ♂.

Fig. 10. The same of *Loemopsylla regis* ♂.

Fig. 11. The same of *Loemopsylla isidis* ♂.

Fig. 12. The same of *Loemopsylla creusae* ♂.

#### PLATE V.

Fig. 1. Genitalia of *Loemopsylla scopulifer* ♂.

Fig. 2. The same of *Loemopsylla erilli* ♂.

Fig. 3. The same of *Loemopsylla niloticus* ♂.

Fig. 4. The same of *Loemopsylla tortus* ♂.

Fig. 5. The same of *Loemopsylla chersinus* ♂.

Fig. 6. The same of *Loemopsylla ramesis* ♂.

Fig. 7. Eighth abdominal segment of *Loemopsylla regis* ♀.

VIII. t.=eighth tergite; VIII. st.=eighth sternite.

Fig. 8. The same of *Loemopsylla pallidus* ♀.

sti.=stigma.

Fig. 9. The same of *Loemopsylla scopulifer* ♀.

#### PLATE VI.

Fig. 1. Terminal segments of the abdomen of *Loemopsylla cheopis* ♀.

Fig. 2. The same of *Loemopsylla divergens* ♀.

Fig. 3. The same of *Loemopsylla isidis* ♀.

Fig. 4. The same of *Loemopsylla nesiotis* ♀.

Fig. 5. Genitalia of *Parapsyllus cocyti* ♂.

Fig. 6. The same of *Parapsyllus corfdii* ♂.

Fig. 7. The same of *Rhopalopsyllus cleophontis* ♂.

Fig. 8. Ninth abdominal sternite of *Rhopalopsyllus cacicus* ♂.

Fig. 9. The same of *Rhopalopsyllus lugubris* ♂.

Fig. 10. The same of *Rhopalopsyllus australis* ♂ from Minas Geraës.

Fig. 11. The same of *Rhopalopsyllus australis* ♂, from Mexico.

#### PLATE VII.

Fig. 1. Genitalia of *Loemopsylla gerbilli* ♂.

Fig. 2. The same of *Loemopsylla mycerini* ♂.

Fig. 3. The same of *Parapsyllus longicornis* ♂.

Fig. 4. Eighth abdominal tergite and seventh to ninth sternites of *Loemopsylla eridos* ♀.

Fig. 5. Eighth abdominal tergite of *Loemopsylla erilli* ♀.

Fig. 6. Ninth abdominal sternite of *Rhopalopsyllus bernhardi* ♂.

Fig. 7. Genitalia of *Rhopalopsyllus bohlsi* ♂.

Fig. 8. The same of *Rhopalopsyllus klagesi* ♂.

Fig. 9. Eighth abdominal tergite of *Rhopalopsyllus klagesi* ♀.

Fig. 10. The same of *Rhopalopsyllus platensis* ♀.

Fig. 11. Ninth abdominal sternite of *Goniopsyllus kerguelensis* ♂.



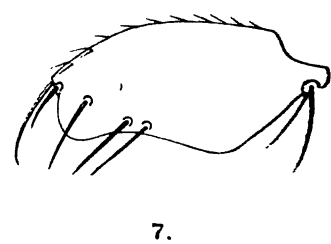
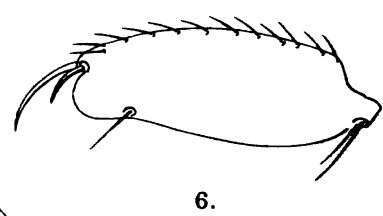
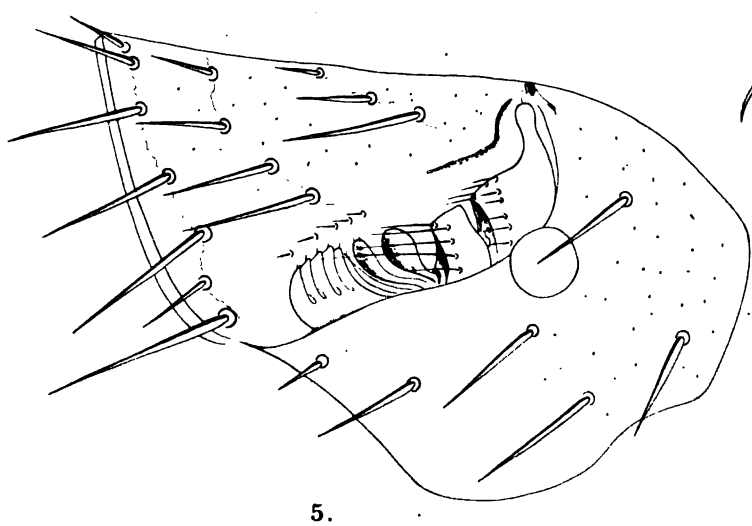
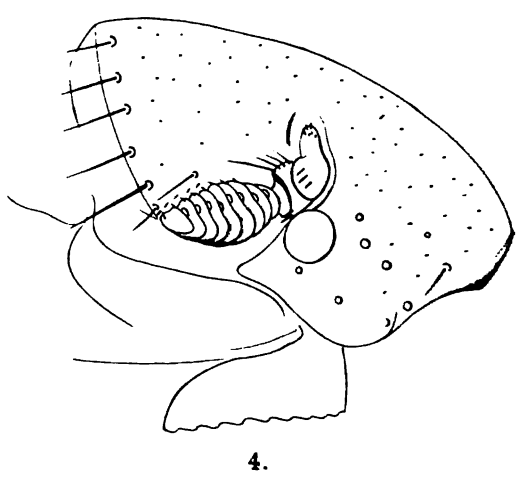
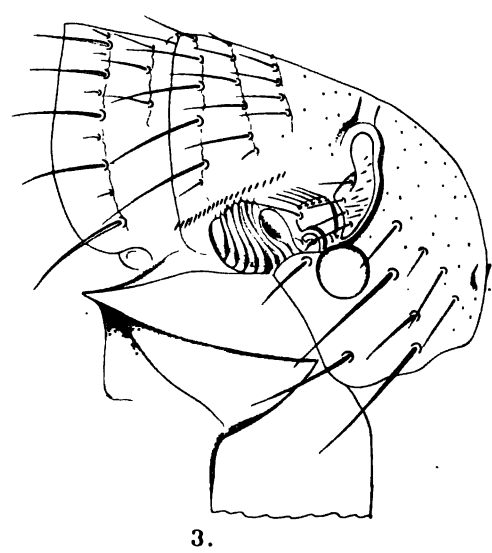
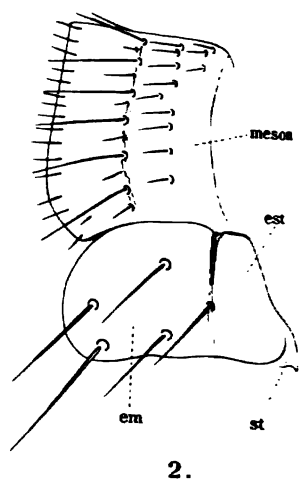
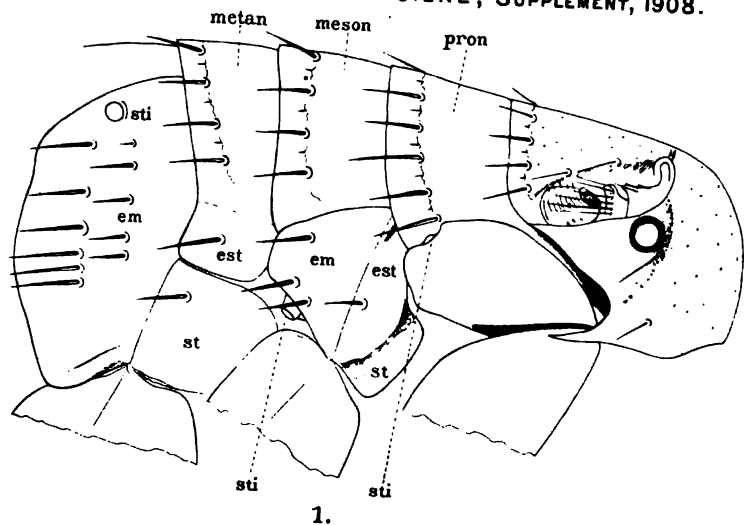
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*Loemopsylla cheopis* ♂.

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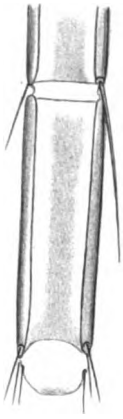


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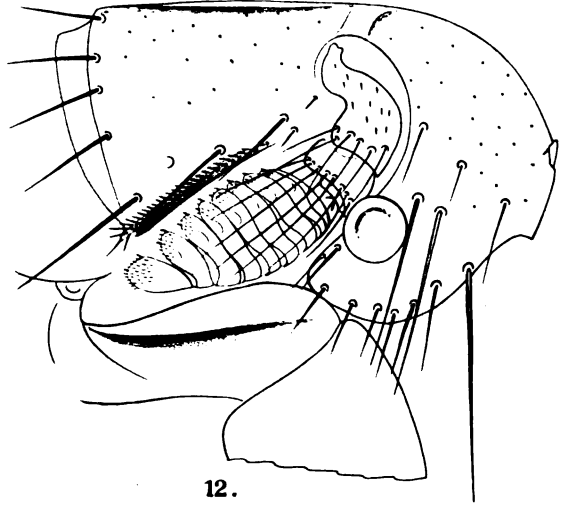




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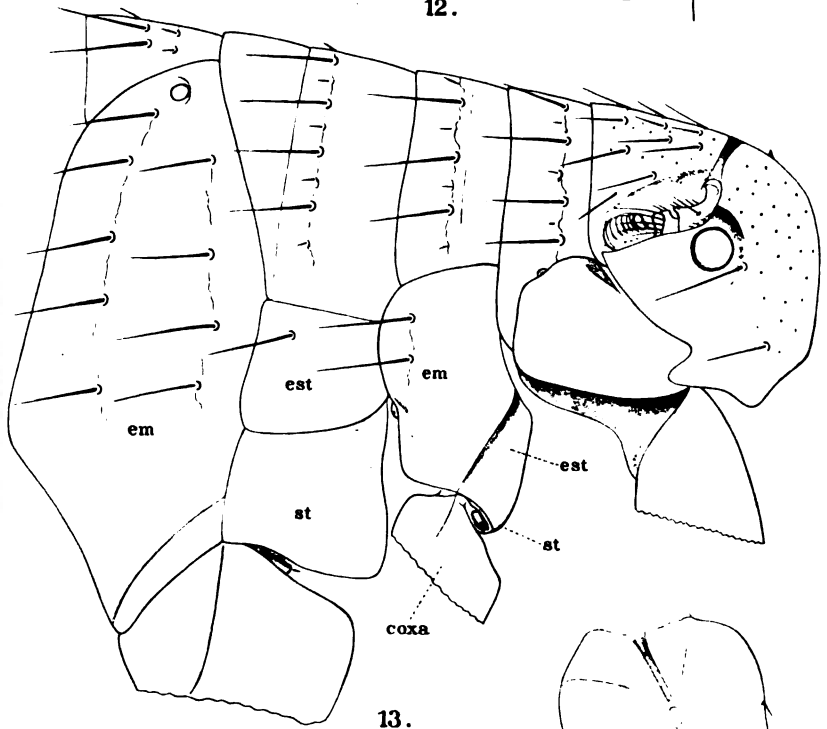
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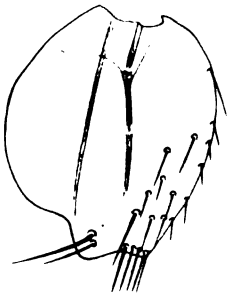
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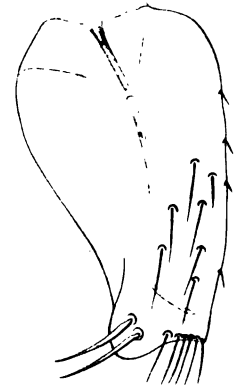
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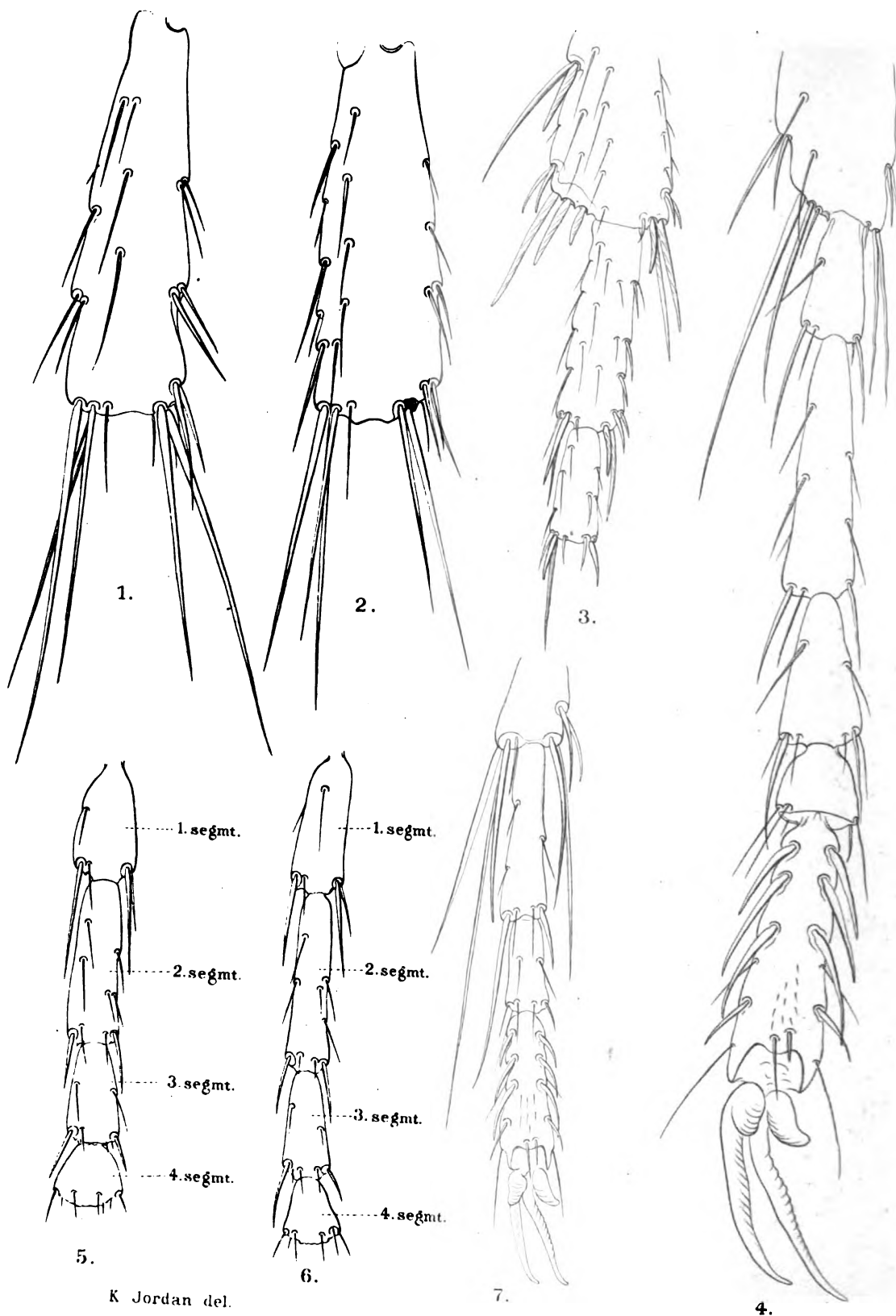
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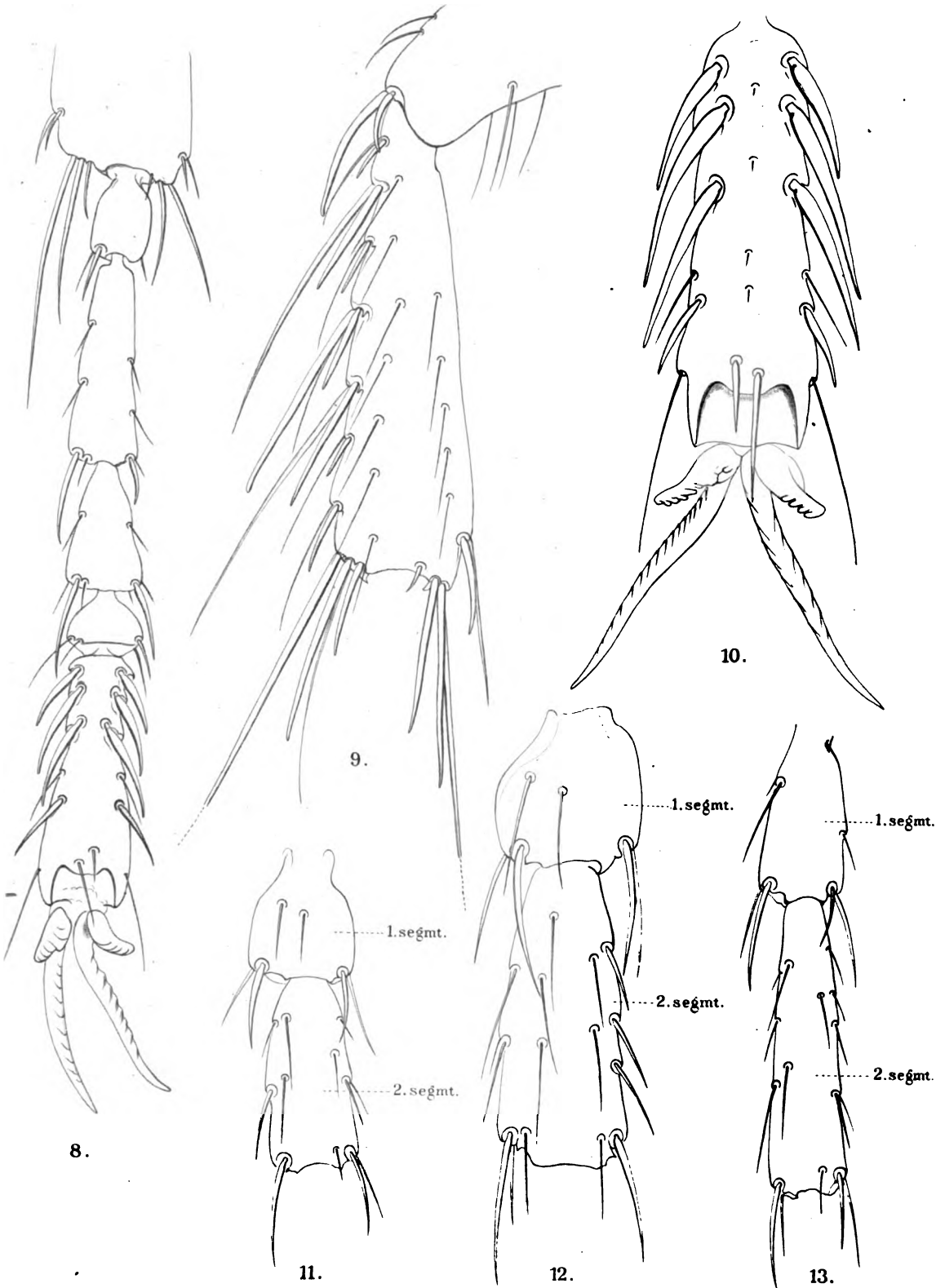






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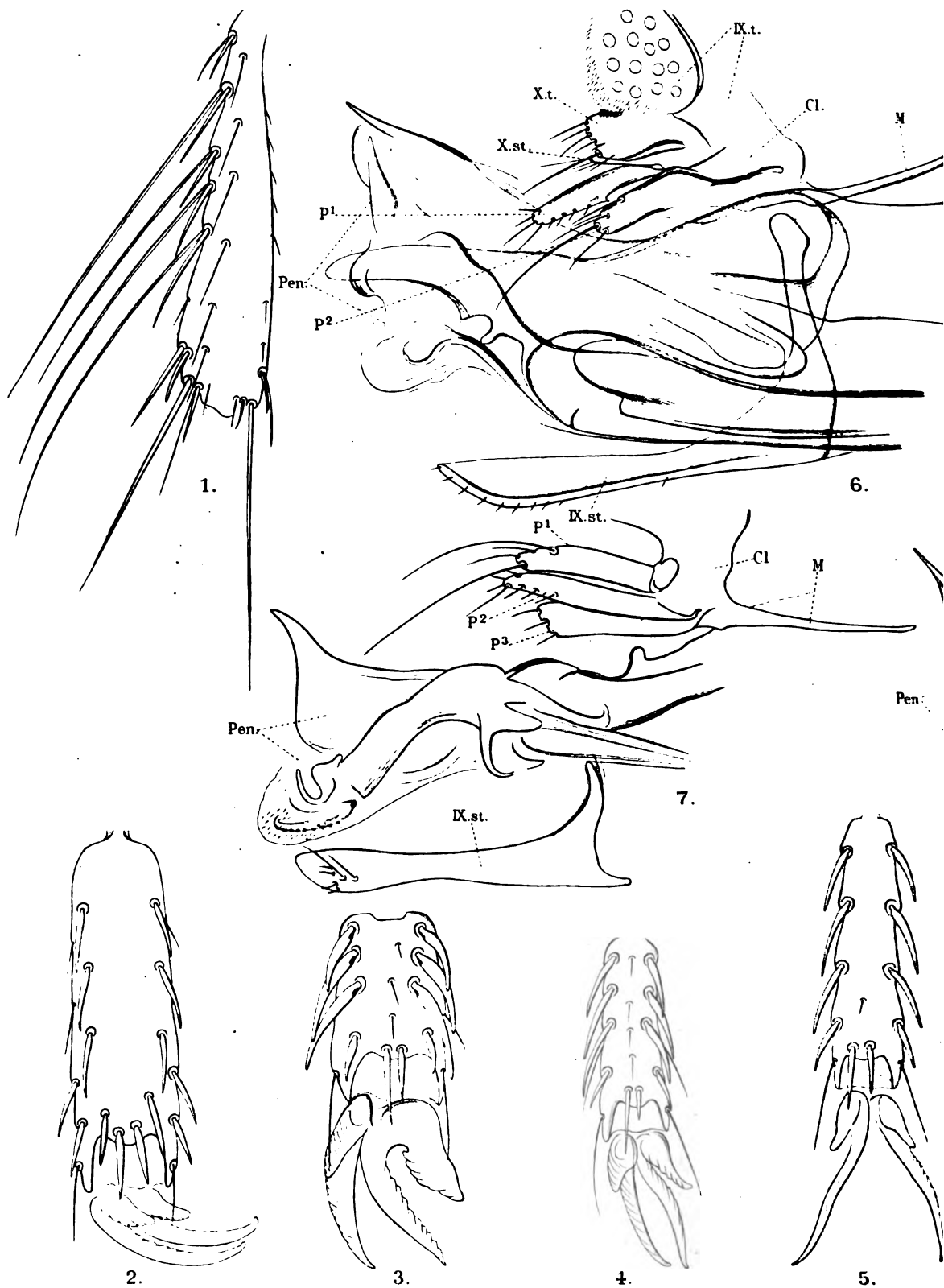
PLATE III.



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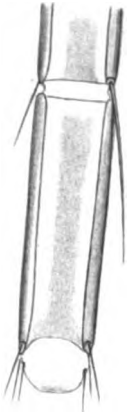


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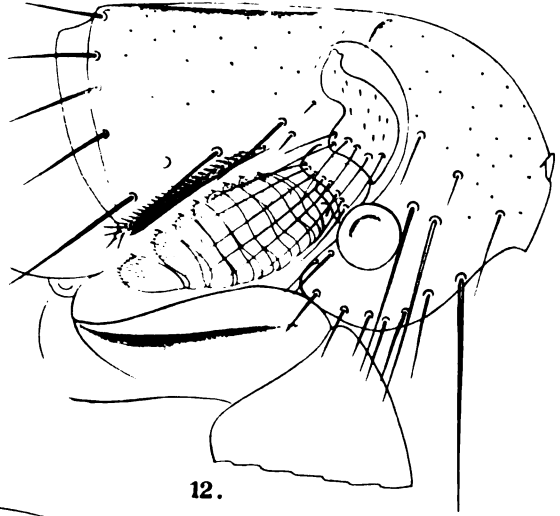




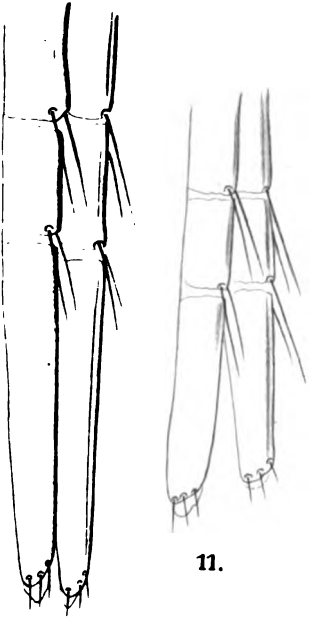
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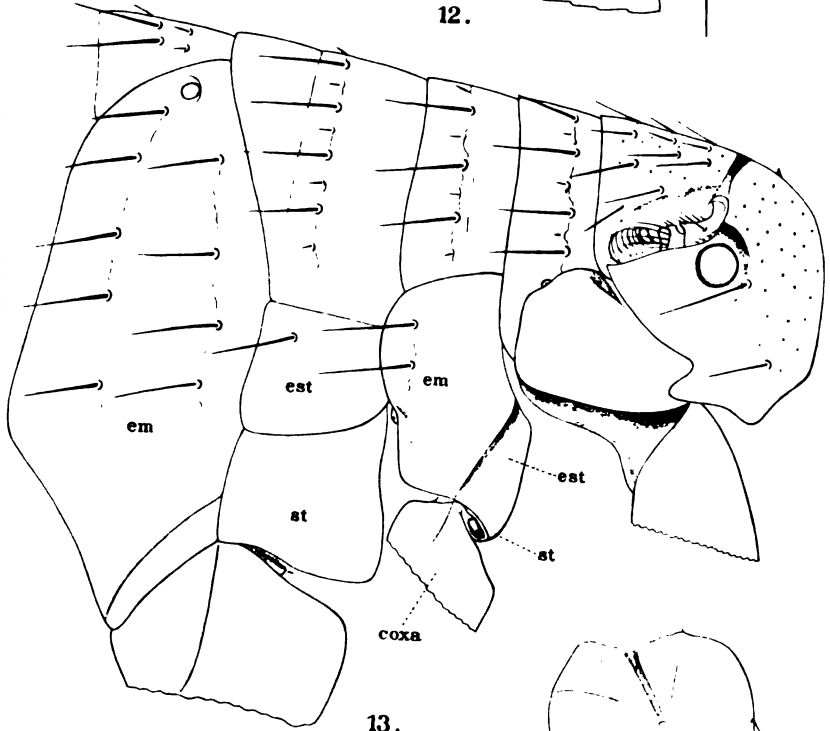


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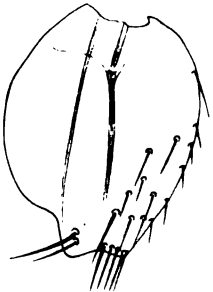


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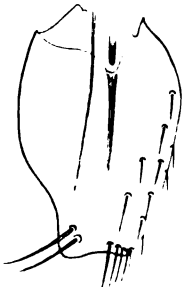
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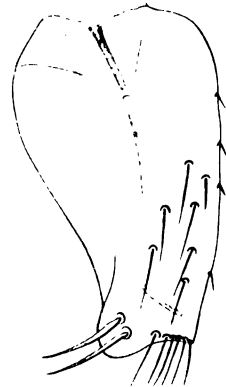
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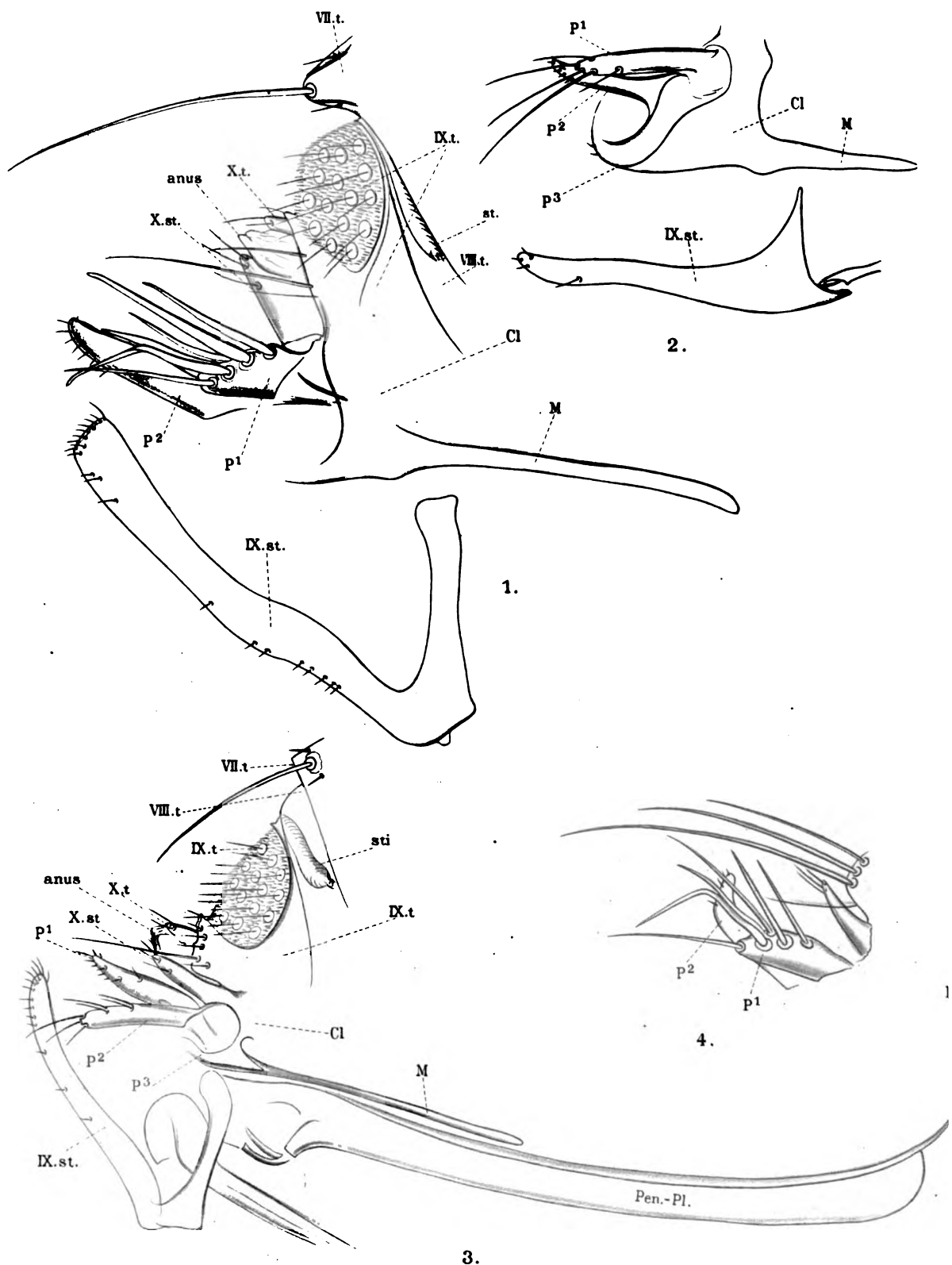
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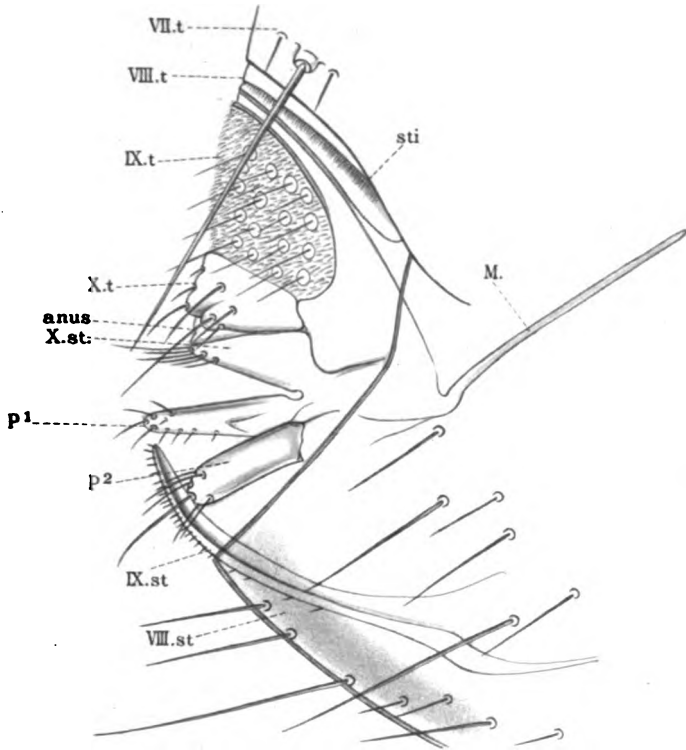




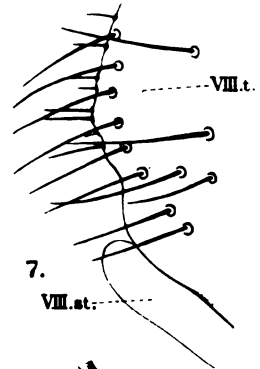


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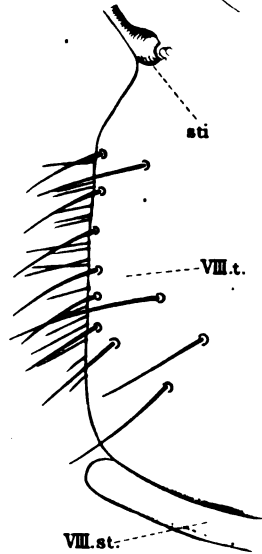
PLATE V.



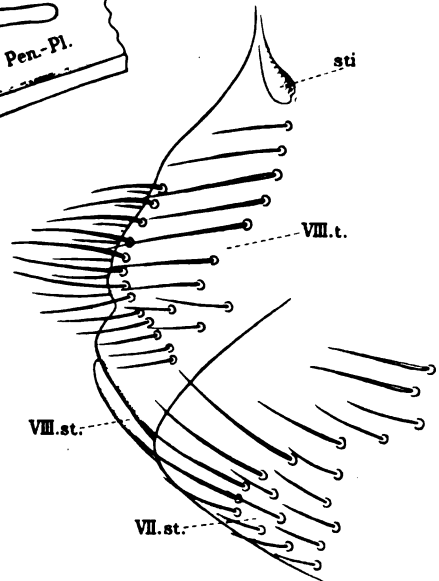
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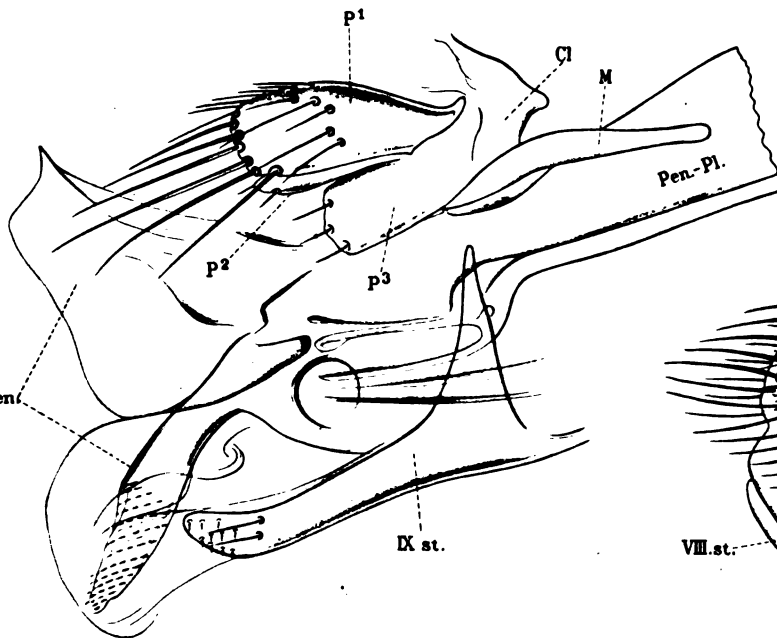
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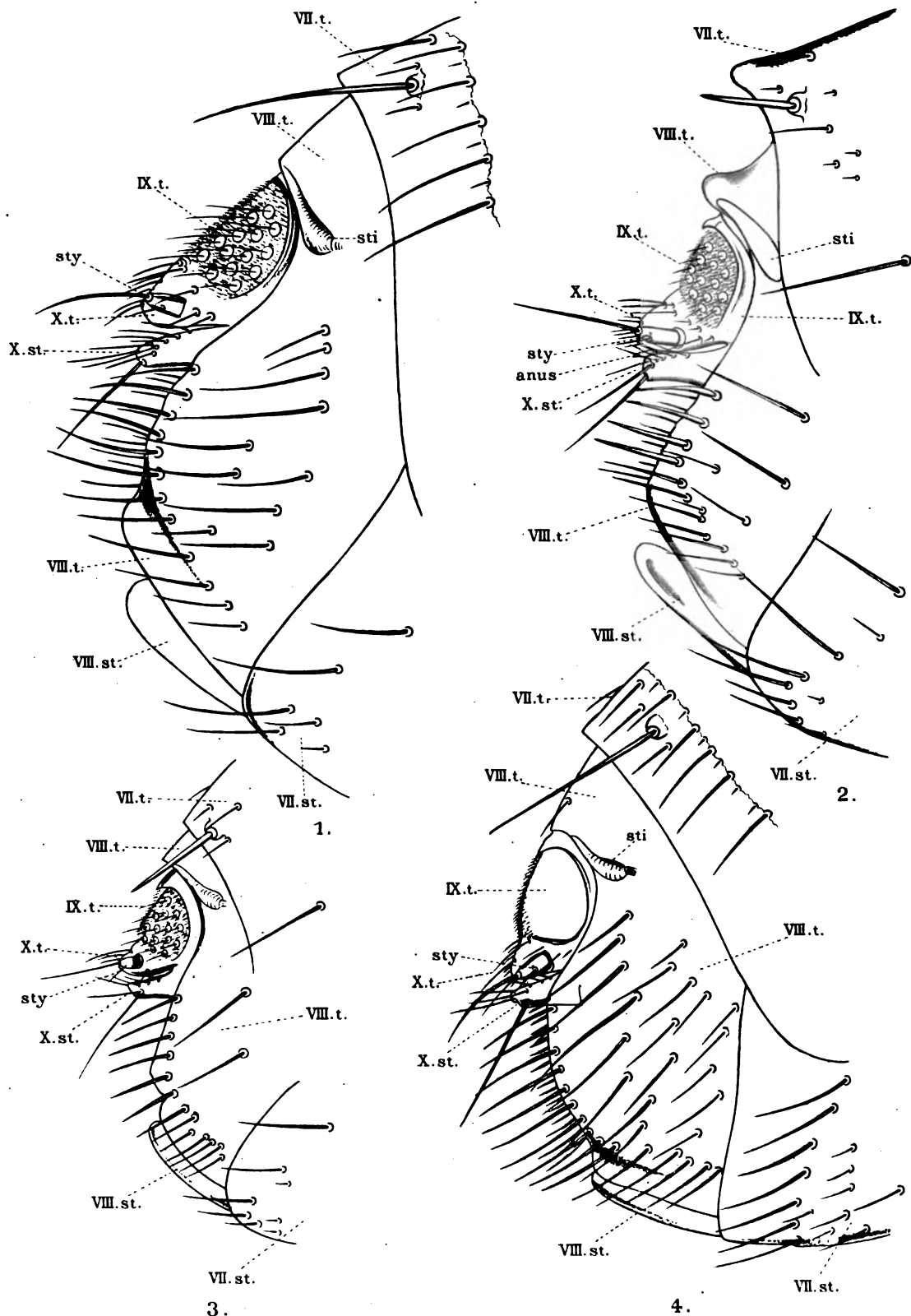
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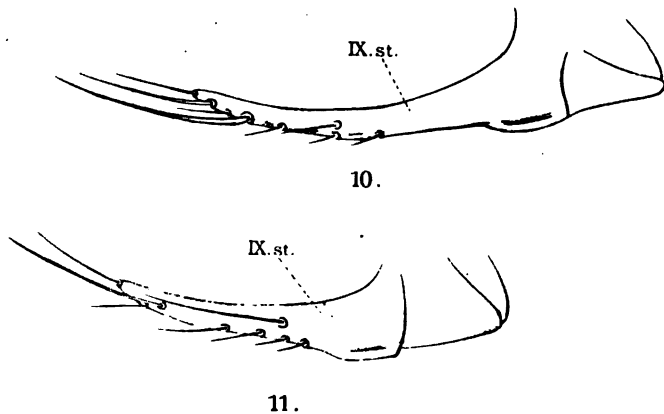
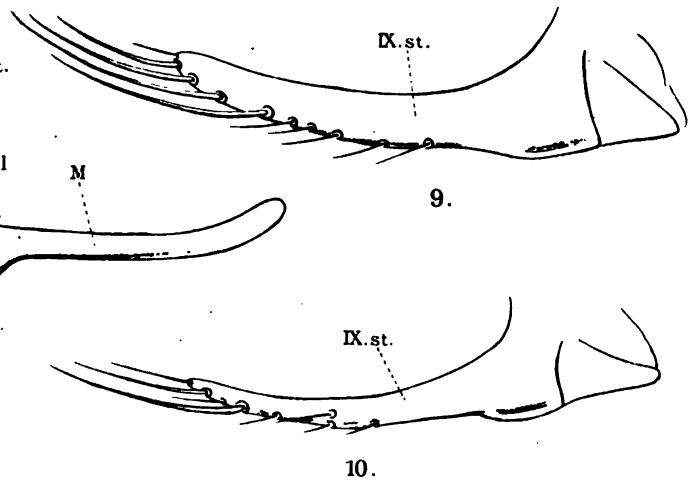
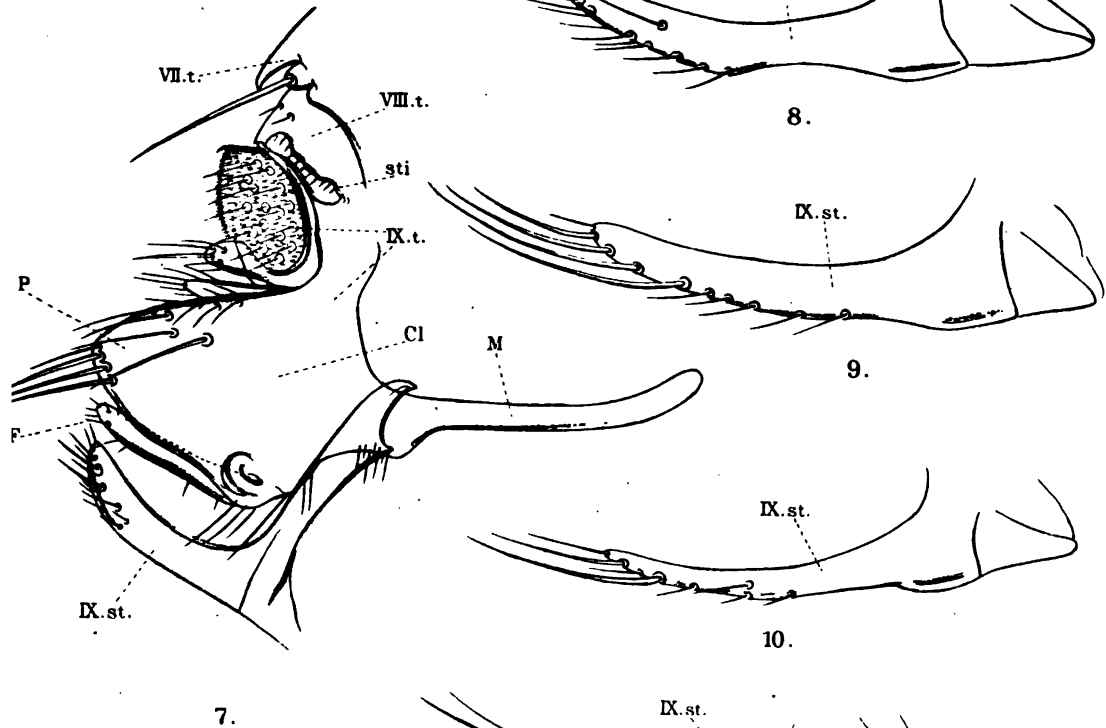
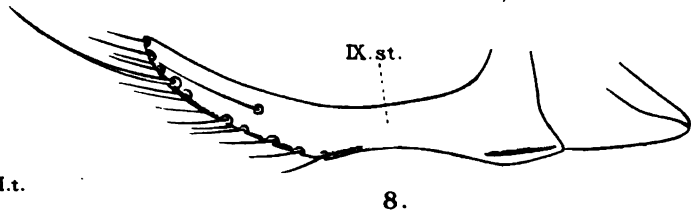
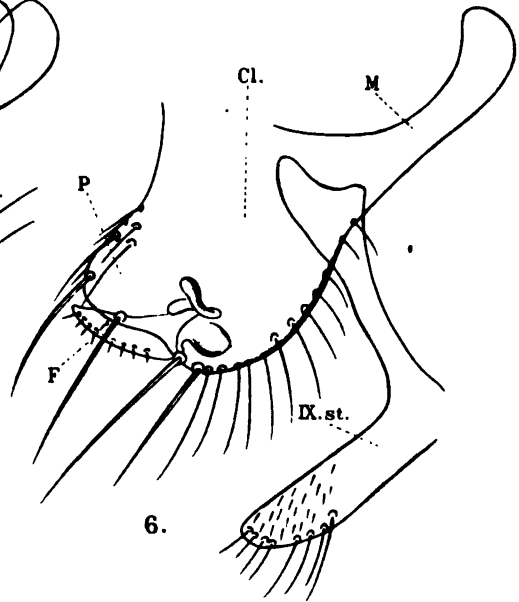
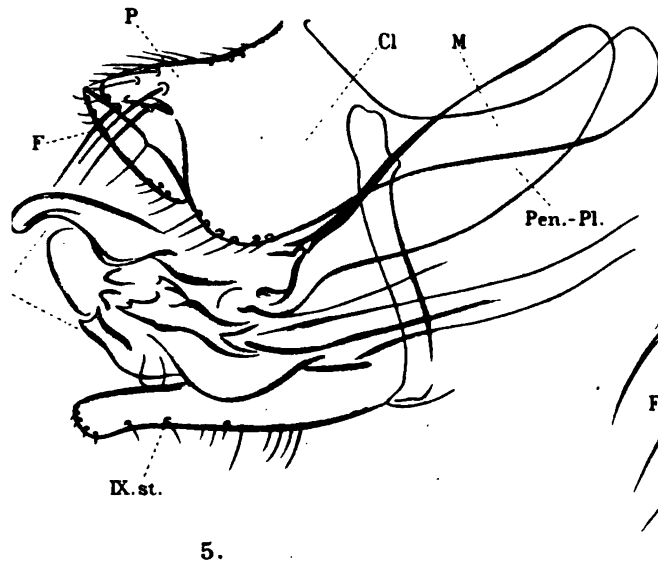






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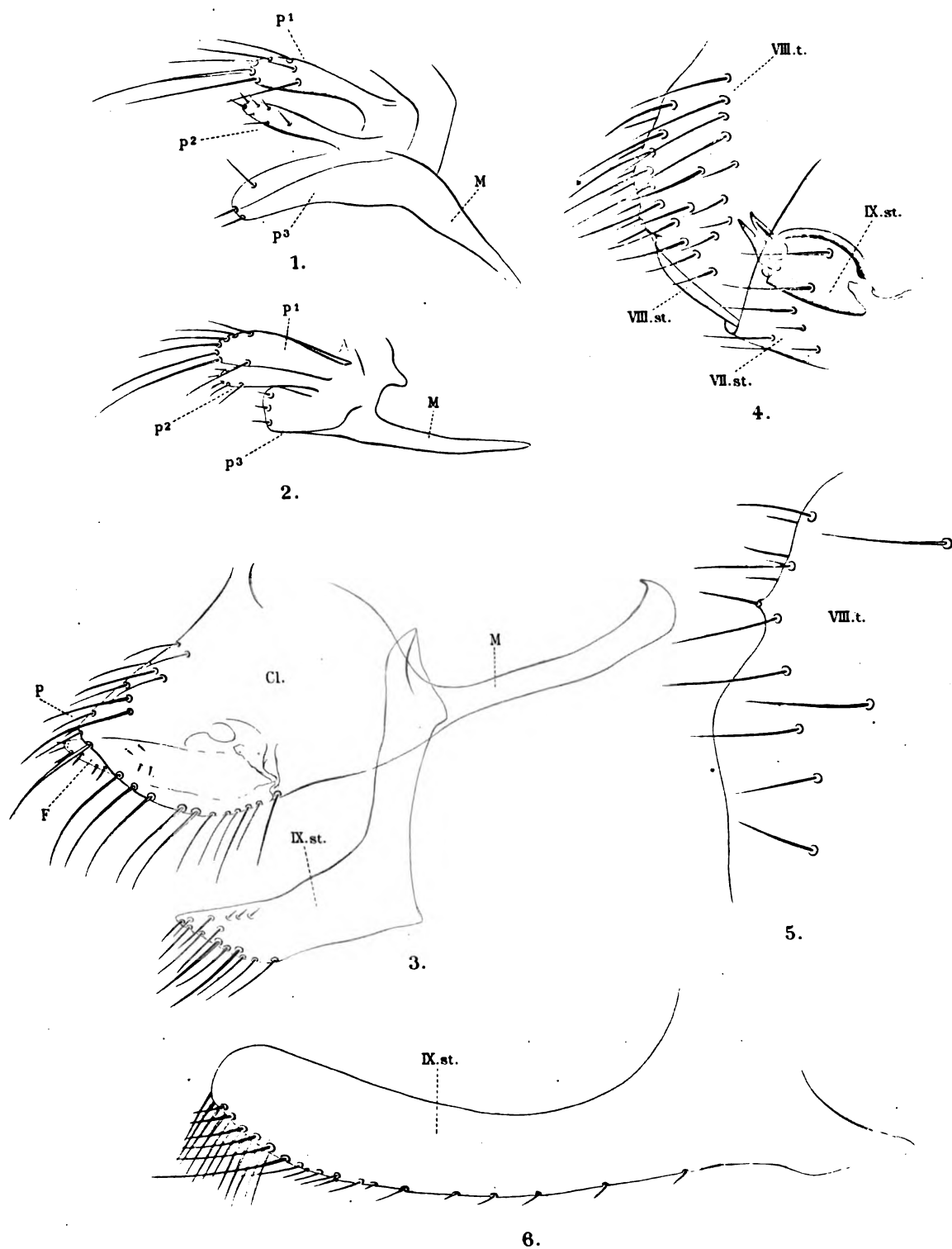


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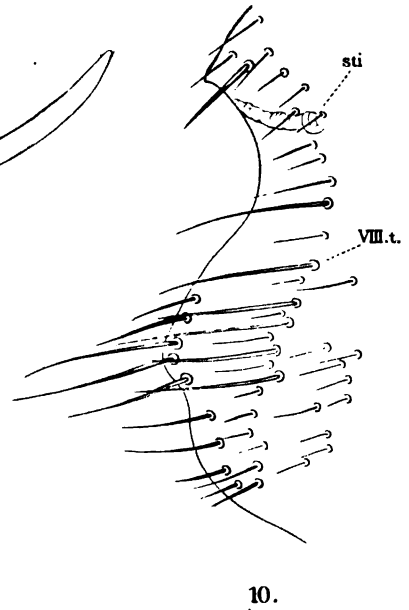
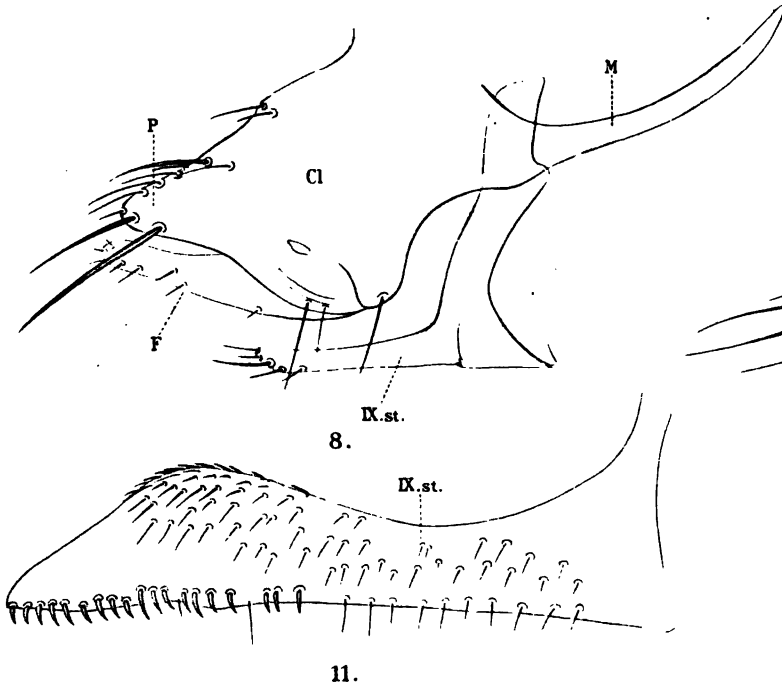
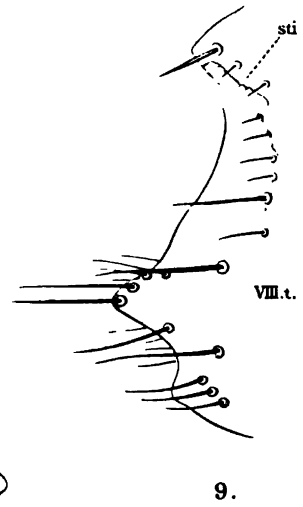
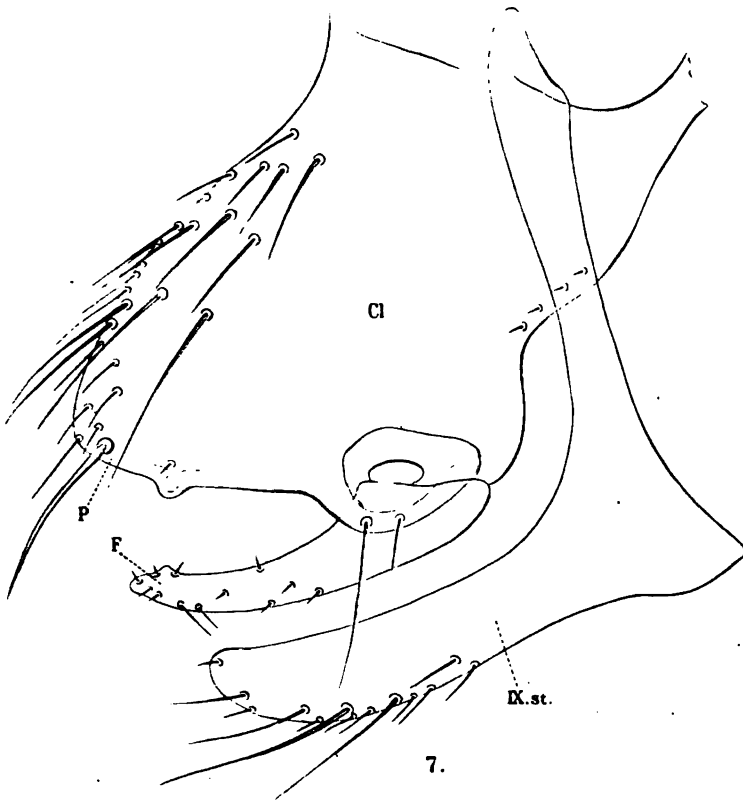
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K. Jordan del.





NOTE ON A LIVER ABSCESS OF AMOEBI-  
ORIGIN IN A MONKEY.

Plate VIII.

By ALDO CASTELLANI, M.D.,

*Director of the Clinic for Tropical Diseases, Colombo (Ceylon).*

SINCE November 1906 I have had in the animal house attached to the Bacteriological Laboratory, a rather large female *Macacus pileatus* (No. 47). This monkey, which had been bought from a villager, had never been used for any experiment: it was kept in the same cage with a monkey inoculated with yaws. In October 1907 the monkey, which so far had been in good health, began to lose appetite, and looked somewhat ill; nevertheless the animal continued to jump about in the cage and play with its companion till a short time before death, on 5. xi. 07. The monkey never had any diarrhoea, and I thought it might have died from a peculiar form of malaria, extremely common in Ceylon monkeys.

*Autopsy*: made immediately after death: lungs and heart normal; heart blood and venous blood did not show the presence of Plasmodia or any other blood parasites; spleen not enlarged, contained no pigment and no haematozoa; intestine to all appearances normal; the mucosa did not show any ulceration nor scars due to earlier ulcerations; contents of colon and rectum semisolid, no blood or mucus; liver somewhat enlarged, a yellowish tumour on the upper surface—which I at first took for a cyst. On cutting, it became clear that it was an abscess the size of a very small nut; the pus was yellowish with a little blood and resembled the pus found in human liver abscesses.

On microscopical examination the pus was found to contain leucocytes, a few red corpuscles, and much detritus. What struck me was the presence of a few slowly moving amoebae. The amoebae were more numerous in scrapings from the walls of the abscess. The amoebae were not present in the intestinal contents which appeared to

be quite normal. I may say at once that (microscopically and culturally) the pus of the abscess, the spleen juice, and the blood from the heart, did not show the presence of any bacteria.

*Description of the Amoeba.* In fresh preparations from the pus of the abscess, the organisms, which were all of large dimensions, appeared moving slowly with the well-known amoeboid movements. The pseudopodia were short, blunt, rather slowly emitted and retracted; the distinction between endosarc and ectosarc was apparently not very marked. The whole body of the parasites appeared vacuolated; but no pulsating vacuoles were present. No nucleus could be distinguished. Some of the amoebae contained red blood corpuscles.

*Stained preparations.* I stained several films from the pus of the abscess by means of the Romanovsky-Leishman method. The amoebae stain bluish, are oval or rounded, and measure 40 to 70  $\mu$ . They occasionally contain red blood corpuscles which appear pinkish or yellowish when stained. In some of the amoebae the nucleus cannot be made out; in others it is small (3 to 6  $\mu$ ), contains some chromatin, is rounded, and occupies an eccentric position. I have not come across encysted or developmental forms.

It is probable, from what has been said regarding the appearances presented by the intestines at autopsy, that this monkey had never had dysentery.

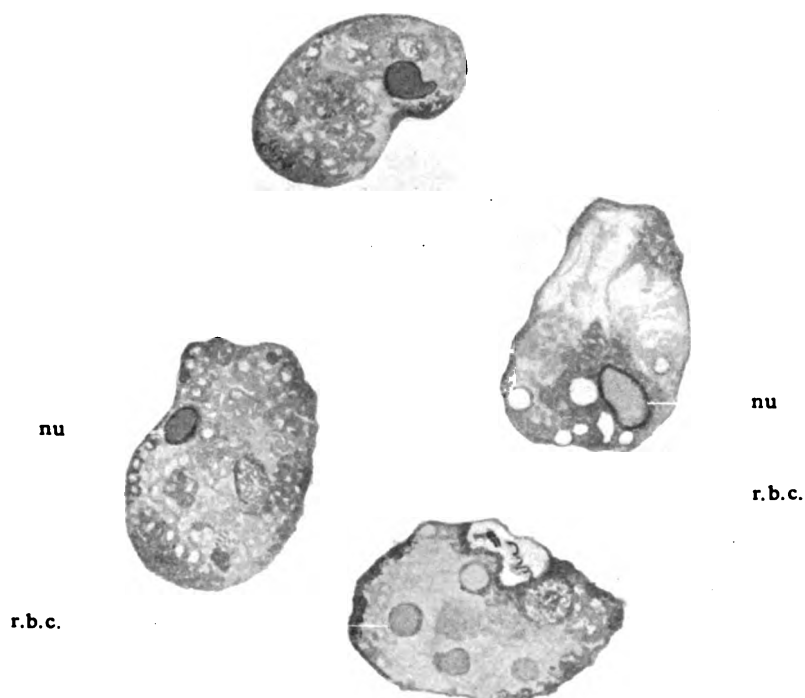
I have seen several cases of spontaneous dysentery among monkeys where the intestinal contents contained amoebae which I believe to be identical with the species found in this liver abscess.

This is the first time, however, that I have come across a liver abscess in a monkey. It is to be noted that amoebae may be frequently found in the faeces of apparently quite healthy monkeys, a fact already noted by several observers; these amoebae, however, are morphologically different as they generally present a rather large, distinct nucleus, and are apparently non-pathogenic.

In conclusion, it seems to me that the abscess of the liver found in the monkey was due to the amoebae I have briefly described, as neither the pus of the abscess, nor the spleen juice, nor the blood of the heart, was found to contain any bacteria; either microscopically or culturally.

For this amoeba I propose the name *Entamoeba nuttalli*, after Professor George H. F. Nuttall.





**ENTAMOEBA NUTTALLI.**

Preparation stained by Leishman's method: nu=nucleus, r.b.c.=red blood corpuscles  
(several of the latter are contained in the parasite at the bottom of the figure).



# ON THE LARVAL AND PUPAL STAGES OF *ANOPHELES MACULIPENNIS*, MEIGEN.

Plates IX and X.

(Continued from *Journ. of Hyg.* Vol. VII. p. 318.)

By A. D. IMMS, B.A., D.Sc.

*Professor of Biology, Muir College, University of Allahabad.*

(From the Morphological Laboratory of the University of Cambridge.)

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## PART II. THE LARVA (*continued*).

### *The Muscular System.*

*The muscles of the head.* In the preceding part of this paper (*Journ. of Hyg.*, 1907, p. 297) some account was given of the muscles of the pharynx and there remain to be considered the rest of the muscles of the head.

These are concerned with the movements of the brushes, the antennae, the mandibles, the maxillae, and the labium.

The muscles of the brushes consist of two pairs, an external and an internal (Figs. 1 and 2). Both pairs arise from the dorsal wall of the head-capsule, and their points of origin coincide with certain symmetrical patches of pigment situated on the surface of the head. Each pair passes forwards to be inserted into the base of the brush of its side. The external muscles (*b'. m'.*) take their origin between and in front of the eyes, and are separated from one another by an interval equal to about half the diameter of the head at its widest part. They converge somewhat as they pass forwards, and each muscle is attached to a chitinous apodeme of the epipharynx. The internal muscles (*b.m.*) arise almost in contact with one another in the middle line of the head just behind the eyes. For the greater part of their course they run almost parallel with one another, and are likewise inserted into a pair of apodemes at the bases of the brushes, slightly above and internal to those of the external muscles.

With regard to the working of these muscles, so far as could be ascertained, they appeared to contract simultaneously with the result that the brushes are depressed so that they become directed backwards and inwards and lie within the space enclosed by the mouth-parts, and their setae come in contact with the surface of the epipharynx. When the muscles are relaxed the brushes regain their normal attitude in virtue of the elasticity of the chitinous framework at their bases upon which the strain is exerted.

The rapid backward and forward motion of the brushes can be readily observed, and though as a rule they work simultaneously, at times one brush may work independently of its fellow.

A third pair of muscles, the *epipharyngeal muscles*<sup>1</sup> (*ep.m.* in Figs. 1 and 2), arise from the middle line on the roof of the head-capsule and just behind the points of origin of the external muscles of the brushes. They pass forwards very nearly parallel to each other and are inserted close together into the membrane of the epipharynx behind the points of insertion of the muscles of the brushes. They appear to function principally as elevator muscles to the roof of the mouth.

At the base of each antenna, along its inner aspect, an *antennal muscle* (*at.m.* in Fig. 1) is inserted. It takes its origin from a strongly chitinised apodeme (the tentorium) arising from the floor of the head. The muscle passes obliquely upwards and outwards in its course to the antenna.

<sup>1</sup> These muscles have already been referred to in the account of the pharynx (*Journ. of Hyg.* 1907, p. 299).

The remaining muscles of the head which have yet to be dealt with are those belonging to the mouth-parts (Fig. 2), and the most prominent of them are the muscles of the mandibles.

Each mandible is moved by a pair of stout muscles, an adductor, which is attached to its inner angle, and an abductor attached to its outer angle. The adductor muscle is the stouter of the two and consists of four separate bands of fibres (*a.m.*). Three of these arise from the wall of the head-capsule a short distance behind and below the eyes, but the fourth band arises separately at a point somewhat in front of the places of origin of the former, and, moreover, it is ventro-lateral in position. The four bundles pass upwards and inwards, converging as they do so, and are inserted into a stout chitinous process from the mandible. The abductor muscle similarly consists of four distinct bands or bundles of fibres (*a'.m'.*), two of these arise close to the three dorsal bands of the adductor while the other two take their origin near to the ventral bundle of the latter.

Attached to about the middle of the base of each maxilla are a pair of muscle bands (*mx.*). If these be traced backwards they are seen to run in close proximity to each other, and their points of origin are situated ventro-laterally on the cranial wall, near to those of the ventral bands of the abductor muscle of the mandible.

The labial plate is provided with a pair of depressor muscles; they are attached on either side near to the base of that organ where it becomes confluent with the ventral surface of the cranium, and converge as they pass forwards to their points of insertion.

Owing to the imperfect transparency of the head-capsule it has not been possible to fully study the movements of the various muscles in the living larva. Their functions, as implied by the names here given, have been largely inferred from a study of their relations to the different parts principally by means of serial sections.

*The muscles of the thorax.* In the thorax there is a well developed system of longitudinal muscles arranged in a dorsal and a ventral series. The dorsal series, or longitudinal tergal muscles, lie a short distance beneath the integument and occupy much of the mid-dorsal area above the digestive canal. The ventral series, or longitudinal sternal muscles, form two groups, one on either side of the nervous system. Immediately in front of the prothoracic ganglia, a transverse muscle band crosses over the nerve cord and unites together certain of the longitudinal sternal muscles. Tergo-sternal muscles are also present, and the most anterior of them is a stout muscle which takes origin from

the dorsal integument in the prothoracic region on either side. This muscle passes downwards and outwards, and becomes attached to the ventral body wall, just external to the imaginal prothoracic leg-bud of its side. About the middle of the thorax a second series of such muscles are present; they consist of several bundles, widely separated from one another, which pass downwards and inwards to the sternal region. In the hinder part of the thorax a third series of tergo-sternal muscles are present; they pass almost vertically downwards on either side between the salivary glands and the cardiac coeca.

The musculature of the thorax exhibits clear indications of an arrangement into three segments, and it is especially well seen in connection with the longitudinal sternal muscles.

In the fully grown larva the developing imaginal thoracic muscles form very prominent objects; they are disposed in a longitudinal and a vertical series on either side of the body. They are very deeply staining and are in the form of short bundles in which are distributed great numbers of minute nuclei, but they do not exhibit any cross striation. The longitudinal series lie above the larval longitudinal tergal muscles and take the form of parallel cords situated on either side of the middle line. The vertical series are situated immediately external to the salivary glands, and are arranged in an anterior and a posterior group on each side.

A number of muscles are also found in the neck, and their action brings about the remarkable rotatory movements of the head. The smaller of these muscles lie for the most part in the neck itself and pass in an oblique manner from one side to the other. There are, in addition, some much stouter muscles, which arise from the prothoracic region and pass up through the neck to be inserted into the base of the head.

*The musculature of the abdomen.* In the abdomen there is no segmentally repeated system of tergo-sternal muscles, such as is present in many insects. The longitudinal muscles<sup>1</sup>, however, are very well developed and are much stouter than those of the thorax, and it is due to the action of these muscles that the larva performs its vigorous eel-like movements as it swims tail foremost through the water. The longitudinal tergal muscles are grouped together on either side of the dorsal vessel, and the sternal series have similar relations with the

<sup>1</sup> Grandpré and Charmoy remark (p. 33), "Les principaux muscles sont les muscles longitudinaux et circulaires de l'abdomen"; this statement, however, is incorrect as there are no circular muscles present.

nervous system (Fig. 3). In the eighth and ninth segments, the disposition of the longitudinal muscles is much modified, and many of the muscles come to lie nearer the digestive canal.

Just in front of each nerve ganglion, a transverse band crosses the nerve cord, and passes along the line of junction of two adjacent segments (Fig. 22).

In the eighth abdominal segment there is a special system of muscles in connection with the supporting skeleton of the spiracles. In order to attempt an explanation of these muscles it is necessary to briefly consider first the structure of this skeleton. From the posterior region of this segment of the body a median spiracular lobe, as it may be termed, projects backwards and very slightly upwards (Fig. 4). It stands out free from the body, and is supported basally by a special chitinised sclerite (*sc.*), which is prolonged on either side into a prominent plate provided with large backwardly directed teeth (*pl.*). The plates of either side are connected together by means of a transverse chitinous band (*t.b.*) which passes beneath the median spiracular lobe, and serves also to maintain the latter in a position so as to project out freely from the general surface of the body. The lobe itself consists of two thin lateral plates (*c.pl.*) which have their free margins curved upwards and inwards, and a central, oblong plate of dark chitin with a curiously sculptured surface (*m.pl.*). At the apex of the lobe the two lateral plates are joined together by means of a median transverse plate (*t.pl.*). The two spiracles (*sp.*) are situated at the anterior end of the median lobe near to the base of the latter and where it becomes joined to the segment. In front of and between the spiracles is situated a moveable transverse plate standing more or less at right angles with the general surface of the body (*f.pl.*). This plate is attached to a stout hollow peg of very dark chitin (*p.*) which projects beneath the integument slightly into the cavity of the animal. To this same peg is also attached the median plate (*m.pl.*) of the spiracular lobe.

There are three paired sets of muscles in connection with the respiratory apparatus as follows: firstly, a pair of longitudinal muscles (*l.musc.*), which take their origin from the median peg (*p.*), and pass directly backwards to be inserted into the spiracular lobe, in the mid-ventral line near to the apex of that organ. Secondly, a series of muscles (*i.mus.*) arise from the ventro-lateral margin of the eighth abdominal segment and, pursuing a backward and inward course, they converge and pass between the two longitudinal muscles just referred

to. The two series cross one another (Fig. 5) and are inserted into the median plate (*m.pl.*) of the spiracular lobe along its middle line. Thirdly, a pair of stout muscles arise one on either side close to the imaginal bud of the gonapophysis of its side and slightly dorsal to it (*o.mus.*). These muscles pass backwards and upwards in an oblique direction, and just external to the longitudinal muscles, and are inserted on either side of the median area (*m.pl.*).

When the animal dives suddenly down into the water, the upper surface of the spiracular lobe is converted into a kind of cup which retains, as a rule, a bubble of air. The contractions of the two sets of muscles (*i.mus.* and *o.mus.*) depress the floor of the lobe, with the result that the sides of the organ are curved inwards, and brought much closer together, and, furthermore, by the action of the longitudinal muscles (*l.musc.*), the anterior and posterior portions of the organ similarly become drawn closer together, and in this way a temporary cup is formed. When the muscles are relaxed, the organ spreads itself out flat, and forms a plate which helps to support the larva at the surface of the water.

In the ninth abdominal segment, a pair of stout muscles take their origin one on either side of middle line of the tergum, and pass obliquely downwards and backwards to become attached, one on either side, to the base of the skeleton which supports the ventral fan of hairs (Fig. 6). By means of the contractions of this pair of muscles the ventral fan, or "tail-fin," can be moved to one side or the other as occasion may demand and, in this way, it appears that the fan of hairs functions as a kind of rudder which steers the animal as it is swimming. If this analogy be a correct one, the two muscles may be regarded as being comparable with the tiller cords attached to the rudder of a boat.

#### *The Fat-body.*

The fat-body is almost entirely confined to the thorax and the first seven abdominal segments. It consists of a parietal layer situated just beneath the integument, a visceral layer lining the body-cavity, and lying between the longitudinal muscles and the gut-wall, and a peritracheal layer which invests some of the principal tracheal trunks. The limits of distribution of each of these divisions vary according to the age of the larvae, and a certain amount of individual variation is also noticeable among specimens of as near as possible the same age.

The visceral layer of the fat-body (Figs. 3 and 9, *f.b.*), although but a thin sheet of tissue, is very conspicuous, as its cells are loaded with a



mass of minute dark olive coloured granules which are very refractive. It is in this layer that metabolism appears to be most active, for the granules are much less abundant in other parts of the animal. A good deal of the greenish colour of the larva is due to the fat-body shining through the transparent integument. The fat-body cells are much vacuolated, and the cell boundaries are only to be distinguished with considerable difficulty. Their nuclei are for the most part small and inconspicuous, and are irregularly stellate in form. The protoplasm of the cells contains, in addition to the granules just mentioned, a number of clear spherical globules which stain very readily with Orange G. The structure of the fat-body agrees in all respects with Berlese's description of the same tissue in the larva of *Culex spathaepalpis* Rond, and he states that the globules are of an albuminoid nature (1901, p. 98, and Tav. V, Fig. 60).

The *sub-hypodermal cells* have the general characters of fat-body cells and, moreover, their protoplasm contains similar granules. As already stated in the previous part of this paper (*Journ. of Hyg.*, 1907, p. 296) Viallanes concludes that they come under the category of this tissue.

The various areas of the fat-body are not to be regarded as being quite separate and distinct from one another. The divisions here adopted have been used because they appear to be convenient for descriptive purposes, and in certain parts of the body one layer merges into the other. The parietal layer is interrupted at each segment, and in this respect shows a metameric arrangement. The visceral layer, however, is in the form of a continuous sheet passing directly from one segment to another.

#### *The Reproductive Organs.*

The rudiments of the gonads differ in form in the two sexes, the male rudiment being short and somewhat globular, while that of the female is relatively longer and fusiform.

The male rudiment in a larva measuring 2.5 mm.<sup>1</sup> in length (Fig. 14) consists of a germinal region, a long anteriorly directed terminal filament or, so-called Müller's thread, and a slight rudiment of the future generative duct. The organ is situated in the sixth abdominal segment. In larvae measuring 5.5—7.5 mm. long, the proportions of these three regions have undergone considerable altera-

<sup>1</sup> The measurements are taken from the tips of the brushes to the extremity of the abdomen, and do not include the dorsal tuft of setae on the ninth segment.

tion, and a long rudiment of the future reproductive duct has been developed. It extends backwards nearly to the posterior border of the seventh abdominal segment (Fig. 11). The duct, at first, takes the form of a solid cellular strand, but becomes hollowed out at a later period. The terminal filament is embedded in the visceral layer of the fat-body (Fig. 13 a.).

In a larva measuring 6.75 mm. long, the female rudiment occupies both the fifth and sixth abdominal segments (Fig. 12), it is more elongated than that of the male of a corresponding age, and shows marked differences in its histological characters. In both sexes the genital rudiment is invested exteriorly by an apparently structureless tunic, and in the later larval periods the central mass of the organ is seen to consist of a number of small cell-clusters, separated from one another by a small amount of interstitial tissue. It is from these cell-clusters that the spermatocytes in the case of the male and the ovarian follicles in the female become developed (Figs. 12 and 13 b.).

But little is known concerning the development of the reproductive organs of Diptera, or other Insecta, during the larval period. Among the Brachycera, some observations are given by Weismann (1864, p. 205, Taf. XIV, Figs. 67—72) in the case of *Musca vomitoria* and *Sarcophaga carnaria*. In the Nemocera, the larval gonads are best known for *Chironomus* (Balbiani, 1885, p. 527, and Miall and Hammond, 1900, p. 135), and *Corethra* (Weismann, 1866, p. 99, Taf. VI). Miall and Shelford also give some brief notes on those of *Phalacrocerus* (1897, p. 35), and Lécaillon for the female rudiments in *Culex* (1900, p. 96).

In *Chironomus* and *Cecidomyia* it is known that the sexual germs develop at an extremely early period in the embryonic life, being formed as polar cells at the surface of the egg before the blastoderm has been developed. How far this precocious development of the sexual germs is at all general among Insects is at present unknown.

### *The Nervous System.*

The nervous system consists of the brain or cerebral ganglia, and a ventral nerve chain of twelve ganglia (Fig. 23).

The brain alters considerably in shape and in the relations of the parts, during the growth of the larva, especially with regard to the optic and antennary lobes. In the very young larva, the optic lobe is separated from the antennal lobe by a wider area than is the case in

the late larva and, as development progresses, they become as it were drawn closer together.

In its general structure, the brain consists of an outer sheath of ganglion cells, which are thickly massed together in the optic and antennal lobes, and an inner central area consisting for the most part of nerve fibres. Paired nerves are given off supplying the antennae and larval eyes, together with a frontal nerve, which expands into a frontal ganglion lying anterior to the brain and just above the dorsal pharyngeal muscles. The antennal nerve passes obliquely outwards, and enters the imaginal antennal bud where it terminates (Fig. 20). According to Miall and Hammond a similar condition is found in the *Chironomus* larva and in their book on that Dipteron (1900, p. 130) they remark that the imaginal antenna encloses the larval antennary nerve.

The ventral nerve chain comprises the sub-oesophageal ganglion, three thoracic ganglia, and a series of eight ganglia in the abdomen. The sub-oesophageal ganglion innervates the mandibles, the maxillae, and the labium, and is to be regarded as a complex ganglionic mass exhibiting no traces of the primitive ganglia of which it is composed, but its paired structure is noticeable in sections (Fig. 32). Histologically, it consists of an external investment of ganglion cells enclosing a central medullary mass of nerve fibres, which is directly prolonged outwards into the lateral nerves and connectives. The ganglion cells are principally congregated along its anterior, ventral, and lateral borders and are much less numerous on its dorsal aspect. A structureless coat of connective tissues invests the exterior of the ganglion. Posteriorly, a pair of stout nerve cords are given off which pass through the occipital foramen and unite with the first thoracic ganglion. The remaining ventral ganglia have a very similar histological structure, and consist of an outer layer of ganglion cells which enclose an inner core of nerve fibres, and the whole is invested by a sheath or epineurium. The connectives, though they are in reality double, appear to consist of a single cord. The thoracic ganglia are situated close together, the intervening connectives being very short, and the abdominal ganglia are placed anteriorly in their respective segments near to the line of junction with the segment in front (Fig. 22). In the eighth segment the ganglion is larger than those of the preceding segments, it is somewhat triangular in form, and it gives off pairs of nerves which supply both the eighth and last segments. It is therefore to be regarded as a fusion of the two primitive ganglia of

those segments. Each ganglion of the ventral nerve cord gives off a pair of principal nerves to its segment, and they pass outwards between the longitudinal sternal muscles and the integument (Figs. 3 and 22).

A system of transverse nerves is also present; a fine nerve runs along the junction between each pair of segments above the abdominal nerve cord, and each is connected with the ventral ganglia by means of median nerves. Owing to lack of opportunity at present descriptions of this and several other important features in the *Anopheles* larva are postponed for a future paper.

#### *Circulatory System.*

The heart is a muscular tube which extends from the posterior margin of the eighth abdominal segment, and passes forwards through the thorax into the head, where it terminates in the neighbourhood of the brain. In the region of the abdomen it consists of a series of consecutive chambers, each being provided with a pair of lateral ostia or inlets and, associated with them, are the alary muscles. In the thorax the heart is much reduced in diameter, there are no alary muscles, and it forms a narrow well-defined tube which is usually termed in Insects the aorta. The latter extends forwards through the occipital foramen and becomes enclosed just behind the brain in an irregular mass of tissue which forms a kind of supporting collar or *anneau de soutien*.

The abdominal portion of the heart consists of a series of eight successive chambers and it expands somewhat at its posterior extremity, the last chamber forming a slight bulbous enlargement. The intimate connection that exists between the tracheal system and the terminal region of the heart has already been referred to (*Journ. of Hygiene*, 1907, p. 311, Pl. V).

The structure of the wall of the heart is entirely muscular, and consists of flattened cells whose protoplasm mainly takes the form of striated fibrillae, which are disposed more or less parallel to one another (Fig. 30). Both externally and internally, the heart is invested by a delicate transparent membrane which is to be regarded as the sarcolemma of the muscle cells (Fig. 29). The striated fibres encircle the heart posteriorly in a slightly oblique manner, but further forwards they become arranged in a longitudinal direction so that their cut ends are only visible in transverse section (Fig. 15). The nuclei of the cells are situated laterally and form noticeable bulgings in the heart wall

(Figs. 9 and 15)<sup>1</sup>. In a transverse section only two of the cells are visible at any given place: they are crescentic in form and are united with each other along the dorsal and ventral lines.

The lateral inlets or ostia are situated in pairs opposite one another on either side of the heart (Fig. 10). Each ostium is formed by a pair of enlarged cells which surround the aperture and are noticeable in stained preparations by their nuclei.

The alary muscles (*al.m.* in Figs. 9 and 10) are markedly cross-striated: each muscle takes its origin from the integument on either side and, as it approaches the heart, it spreads out fanwise and divides and sub-divides so as to form a network of fine fibrillae. These fibrillae are arranged in the form of a dorsal and ventral sheet (Fig. 9), and in the space or interval left between them are situated the pericardial cells. In addition to the alary muscles, the heart is maintained in position by means of delicate strands of connective tissue placed at different points.

The pericardial cells (Figs. 9 and 10) are placed laterally in relation to the heart, and form a chain of cells arranged in a linear series on either side. The cells are disposed with their long axes parallel with the length of the body, but the chains are not continuous throughout their length, numerous gaps or breaks in the series being present. They extend right through the abdomen to about the middle of the eighth segment. The cells contain several nuclei and their protoplasm is much vacuolated. In *Sayomyia* the pericardial cells were described by Wagner (1835) as "piriform bodies" and later by Dogiel as "apolar nerve cells" (1877, p. 9). In the anterior part of the thorax, and situated close to the heart on either side, is a longitudinal cord of multinucleated cells (Fig. 15). These two cords occupy very much the same position as the pericardial cells in the region of the abdomen and very possibly they are structures of a similar nature.

The aorta is a forward prolongation of the heart through the thorax into the head. It has stout walls, and the nuclei of the cells are disposed with long axes parallel with the length of the tube. No alary muscles are present. The aorta passes forwards between the two dorsally placed cardiac coeca, and lies just over the oesophagus but, as it approaches the occipital foramen, it comes to lie nearer the dorsal integument. Just behind the brain, it becomes supported by an irregular mass of cells which envelops it both ventrally and laterally.

<sup>1</sup> In the larva of *Sayomyia* the nuclei project a long way inwards and were termed the "stalked cells" by Leydig.

This structure is a kind of supporting collar (*anneau de soutien*) and is composed of cells which have no definite outlines and are in some cases vacuolated. It is invested by a connective-tissue coat, and is maintained in position by some delicate muscle fibres arising from the head-capsule. Furthermore, it is also attached by means of fine membranous strands both to the connective-tissue coat of the oesophagus and, a little further forwards, to the commissure uniting the two cerebral ganglia (Figs. 17, 18 and 21). Posteriorly, the organ bifurcates into a pair of cellular prolongations (Fig. 16); while anteriorly it extends a short distance forwards between the cerebral ganglia. A similar structure has been described by Weismann (1864, Pl. VIII, Fig. 8) in the larvae of Muscids and also by Lowne (pp. 88 and 91). It is, furthermore, found in the larvae of the Syrphidae (Kunckel de Herculais, 1875, Pl. XIII, Fig. 5), of the Tachinid *Thrixion* (Pantel, 1898, p. 170) and of *Sayomyia* (Dogiel, 1877, Fig. 1). In the latter it is placed behind the brain as in *Anopheles*, but in *Thrixion* it lies for the most part above the cerebral commissure and in a more forward position. Dogiel states that in front of the collar the aorta divides into two lamellae, one of which extends under the brain towards the eye, and the other is more dorsally situated. At the point where the two lamellae arise the aorta opens freely into the haemocoelic space of the head. In *Anopheles* the further course of the aorta was found to be very difficult to follow, but it seems to divide into two lamellae as in *Sayomyia*, one of which is closely applied to the under surface of the cerebral commissure, and the other appears to be in intimate association with the dorsal surface of the anterior prolongation of the *anneau de soutien*.

The dorsal lamella in *Sayomyia* and *Anopheles* appears to be the homologue of the *gouttière susoesophagienne* described by Pantel in the heart of the larva of the Tachinid genus *Thrixion*.

#### *The Oenocytes.*

Oenocytes are present in the larva of *Anopheles* and belong to two varieties—the large and the small. The large oenocytes (Fig. 26) are segmentally arranged in clusters, and are present in each of the first seven abdominal segments, but were not observed either in the eighth or ninth segments, and they are likewise wanting in the thorax. In each of the segments where they occur, they consist of two pairs of very large cells on either side, which are situated a short distance beneath the hypodermis and towards the posterior margin of the

segment. Each cluster of these cells is ventro-lateral in position. The cells are rounded or somewhat oval in form with a granular and very deeply staining cytoplasm, and each cell is bounded by a clearly defined membranous investment. Numerous large vacuoles are generally present and when viewed in sections they give the cells the appearance of being penetrated by intra-cellular canals. Each oenocyte has a very prominent nucleus containing chromatin strands situated towards the periphery, and a deeply staining nucleolus is also present.

The small oenocytes are very numerous and, moreover, have no definite arrangement though they are sometimes found in pairs (Figs. 22 and 27). They occur just beneath the hypodermis in the neighbourhood of each group of the larger oenocytes, and are mainly situated anterior to the latter, but they also occur in some numbers along the floor of each segment on either side of the nerve cord. They have a similar marked affinity for staining reagents and frequently each contains a clear vacuole (Fig. 22). A nucleus with a central nucleolus is present, and these cells are easily distinguished from the surrounding fat-body by their definite outlines and their staining properties. They occur in the first seven abdominal segments, a few are also present in the eighth but none were to be discovered in the last segment.

The embryonic development of the oenocytes has been studied by Graber, Heymons, Heider, and Wheeler. They arise by a proliferation of the ectoderm just behind the tracheal invaginations, and similar metameric cell-clusters have also been observed by Tichomiroff in the embryo of the "silkworm."

Wielowiejski (1886) was the first to devote special attention to these cells and the name oenocyte is due to him. He pointed out their resemblance to the blood corpuscles, to the fat-body, to the pericardial cells, and to the light-producing tissue of the phosphorescent organs and classified these different elements together under the category of blood tissue. In his paper he describes the oenocytes in various orders of Insects, but unfortunately his account is not accompanied by any figures. Among the Culicidae, he refers briefly to their occurrence in the larvae of *Sayomyia* (*Corethra*) *plumicornis* and *Culex pipiens*. In the former he mentions that they are of two kinds, the large and small oenocytes, and that they are situated between the cells of the outer layer of the fat-body. They are restricted to the abdominal region and are collected together into segmentally repeated groups (1886, pp. 516—17). In the *Culex* larva he describes their occurrence as follows: "Die kleinen Oenocythen liegen auf der der Leibeshöhle

zugewandten Fläche des äusseren Lappens<sup>1</sup> festgeklebt, oftmals bis 10 und mehr auf einem Querschnitte und zeichnen sich durch grosse Tinctionsfähigkeit vor sonstigen aus. Grosse Oenocythen sind auch in Gruppen zu vier bis fünf segmentweise im Abdomen angeordnet, liegen aber eben so wie die kleinen auf der Innenseite des die Leibeshöhle auskleidenden Fettkörperlappens befestigt."

Oenocytes have been discovered in a number of dipterous larvae, and it is extremely probable that they are of general occurrence throughout the order. In *Chironomus* they have been described by Wielowiejski (p. 515) and Miall and Hammond (1900, pp. 40—42). The large oenocytes are restricted to the abdomen, and consist of a group of five very large cells on either side in the first eight segments. The fifth cell in each group lies in front of the cluster of four cells and, moreover, differs from them in containing two nuclei, viz. a large central one and a small one situated at the periphery. The small oenocytes are very numerous and are found towards the ventral surface of the metathoracic and abdominal segments, just beneath the integument. Oenocytes are also present in the larvae of *Tipula oleracea* (Wielowiejski), *Phalacrocerca replicata* (Miall and Shelford, 1897, pp. 352), *Simulium* (Vaney, 1202) and other Nemocera.

Among the Brachycera they appear to have been very little studied. Wielowiejski (p. 520) states that in this group of Diptera they present the characters of the large oenocytes of *Chironomus*, and differ chiefly in being more numerous, and in having a more intimate connection with branches of the tracheae. They have been carefully studied, however, by Pantel (1898, p. 210) in the Tachinid, *Thrixion halidayanum*. In the larva of this fly they are collected into a ventral and a lateral group on each side, in the seven posterior segments of the body. Each group contains as a rule 3—6 cells, which are soldered together into a "chapelet." Pantel puts forward the suggestion that possibly one of the two groups in *Thrixion* is the homologue of the binucleated cell in *Chironomus* and that the latter is in reality the rudiment of a colony of uninucleate cells.

Among the Pupipara, according to Berlese (1901, p. 145) in *Melophagus ovinus*, the oenocytes are disposed in metameric groups in the larva but, at the commencement of nymphosis, they multiply and are found in numbers among the cells of the fat-body, and subsequently disappear at the time when the Malpighian tubes are developing.

<sup>1</sup> i. e. the "Fettkörperlappens."



Oenocytes have been found in almost all orders of Insects, but practically nothing is known regarding their function. Wielowiejski believes that they secrete some unknown substance into the blood, and Anglas (p. 405) is of a similar opinion. On the other hand, Pantel concludes from the fact that they absorb methylene blue very rapidly, like the cells of the Malpighian tubes, that their function is that of excretion. Berlese (in *Melophagus*) concludes that they are urinary or excretory cells from the fact that they become free during nymphosis, at a time when the Malpighian tubes are non-functional, and that they work their way among the cells of the fat-body, &c. in order to remove the products of metabolism from them. After a while the oenocytes disappear, and it is at this period that the Malpighian tubes are fully developed.

Koschevnikov (1900) from his studies on the honey bee also concludes that they are urinary cells.

### *The Imaginal Buds.*

The larva of *Anopheles* forms an excellent subject for the study of the imaginal buds since they occur in this type in a very generalised condition. It is, however, beyond the scope of the present paper to deal with this subject in any detail as it needs a much more prolonged study than I have been able to give to it.

Swammerdam appears to have been the earliest observer to correctly interpret certain of the imaginal buds, and he discovered those of the wings and legs. *Culex* was among the types he studied, and since his time Weismann (1866) has studied the imaginal buds of the larva of *Sayomyia*. A few unpublished observations on those of the former genus were made by Hurst, and are mentioned by Miall and Hammond (1892), and Thompson has described the buds of the mouth-parts in that same genus (1905). I am not aware that there are any further accounts which deal with these structures among larval Culicidae.

I. *The imaginal buds of the head.* The largest and most prominent of the head buds are those belonging to the antennae. The *antennal buds* (Figs. 2 and 20) are placed at the bases of the larval antennae, but are not in any way enclosed by the latter and are, moreover, rather deeply situated within the head. In an *Anopheles* larva about three-fifths grown each imaginal antennal bud is a well developed structure and, owing to the growth of the organ within a limited space, the basal joints are somewhat folded and telescoped into one another. As the appendage enlarges, its outer sheath or peripodial membrane

(*pp.m.*) becomes a good deal stretched and is reduced to a thin layer of tissue. At this stage the first three basal joints are already developed but the remainder of the organ is still unsegmented. In a fully grown larva the second joint is greatly enlarged, especially in the male, and its cellular wall is very much thickened so as to almost obliterate the cavity of the joint. This condition is a stage in the development of the prominent antennal sense organ known as Johnston's organ. Although the antennal buds are situated the deepest below the hypodermis of all the imaginal buds, they retain their connection with the surface of the head by means of a short canal which is the persistent mouth of the early invagination.

*The buds of the mouth-parts.* The earliest indications of the formation of the future mouth-parts are noticeable as special thickenings of the hypodermis, situated at localised points beneath the cuticle of the larval mouth-organs. Of these buds, the rudiments of the future labium, labrum, and maxillary palpi alone attain the condition of imaginal folds prior to pupation. Those of the mandibles and the maxillae remain in the condition of simple, hypodermal thickenings, but are easily recognisable on account of their great affinity for staining reagents. The invaginations of the first mentioned series of buds are superficial in position, and they maintain their communication with the surface by means of wide mouths or openings. The bud of the labrum is unpaired and situated in the middle line. It commences as a hypodermal thickening on the roof of the head and gradually elongates and comes to lie some distance backwards. Those of the maxillary palpi are small structures, and are situated at the bases of the same organs in the larva. The labial bud is extremely prominent (Fig. 31) and takes the form of a paired structure projecting from the bottom of a wide pocket. It is the earliest to appear of the imaginal buds that go to form the mouth-parts. Its first indication in the young larva is in the shape of a thickening of the hypodermis underlying the anterior border of the labial plate. In longitudinal sections through the head of a fully grown larva, the labial buds form a prominent pair of hollow pointed projections which are confluent only at their bases.

II. *The thoracic buds.* In the thoracic region three successive pairs of buds are present on either side belonging to the pro-, meso-, and metathorax respectively. They are disposed in a dorsal and ventral series, the latter eventually giving rise to the three pairs of legs, while of the former series the first pair form the pupal respiratory siphons, the second pair the future wings, and the third pair the halteres. All

the thoracic buds are relatively very large in size, and are superficial in position, lying just beneath the integument. At first they each have the structure of simple buds (Fig. 19) of thickened hypodermis, enclosing a small amount of "mesenchymatous" tissue, but, by the time the larva has become fully grown, they are seen to exhibit a high degree of specialisation. The dorsal prothoracic buds have become the completely formed respiratory trumpets; those of the mesothorax are highly complex organs (Fig. 25), and are much folded and plicated. They already exhibit a wing-like character, the hypodermal cells being greatly drawn out at right angles to the surface of the bud, assuming a pillar-like form, and the upper and lower hypodermal layers meet and fuse with one another. In places they are excavated into hollow tube-like spaces which are forerunners of certain of the future nervures; the dorsal metathoracic buds remain in a more generalised condition, being the smallest and least advanced of the series. The leg-buds are at this period long tube-like organs which are bent upon themselves.

III. *The imaginal buds of the abdomen* comprise a dorsal and ventral pair situated on either side, near the hinder extremity of the body. The dorsal pair of buds form the pupal tail fins; they are placed one on either side of the eighth abdominal segment, and lie within the cavity of the supporting skeleton of the larval spiracles (Figs. 7 and 8). They exhibit very much the same structure as the buds of the wings, the hypodermal cells being enormously drawn out at right angles to the surface of the organ and the protoplasm reduced to narrow strands in which are distributed extremely small nuclei. They are greatly folded, on account of being confined within a limited space, and investing the free surfaces of the folds is a well developed cuticle. They are situated within the lateral plate (*sc.*) of the spiracular skeleton, and project freely backwards reaching to the transverse chitinous band (*tb.*), and thus lying beneath the spiracular lobe. They appear to differ from the other buds in being directly formed by modification of the hypodermis, without the latter being previously invaginated to form a pocket, and from the bottom of which the buds arise usually as finger-like evaginations. On this account, the buds of the pupal fins are not enclosed within an outer wall or peripodial membrane.

The second pair of buds (Fig. 28) are ventral in their position, and placed close to the points of origin of the outer vertical muscles (*o.mus.* in Fig. 5) of the spiracular apparatus. These buds like the rest lie immediately beneath the hypodermis, and maintain a free communication outwards by means of the mouth of the original

invagination. They are destined to form the gonapophyses or accessory copulatory organs of the imago.

The following table shows the general conditions of the imaginal buds among the Diptera, and the relationships of the Culicidae in this respect to other forms.

*Imaginal buds of larval origin.*

A. Imaginal buds superficial in position being situated just below the hypodermis; the primitive invaginations remain permanently open.

1. *Anopheles*; *Culex* (Miall and Hammond ex mss. Hurst). Head folds shallow, not extending into larval thorax.

2. *Corethra* (Weismann); *Simulium* (Weismann, Vaney); *Tanypus* (Vaney). Intermediate between 1 and 3.

3. *Chironomus* (Miall and Hammond); *Ceratopogon* (Miall and Hammond); *Stratiomys* (Vaney)?; *Cecidomyia* (Marchal). Head folds extend into larval thorax.

*Imaginal buds for the most part of embryonic origin.*

B. Thoracic buds superficial in position, cephalic buds as in C.

*Melophagus* (Pratt). Possess an additional pair of ventral cephalic buds not discovered in other Diptera.

C. Imaginal buds deeply situated below hypodermis, only maintaining their connection with the latter by means of a thread-like pedicle. Cephalic buds situated in larval thorax.

*Muscids* (Weismann); *Anthomyia* (Ganin); *Volucella* (Kunckel d'Herculais); *Eristalis* (Wahl); *Gastrophilus* (Vaney).

*The Eyes.*

In newly hatched larvae, and in larvae measuring up to 2·75—3·5 mm. in length, a single oval deeply pigmented eye-spot is present on either side of the head. These *larval eyes* ("Nebenaugen") are placed about half way between the neck and the bases of the antennae, and each has its long axis disposed transversely to the length of the head (*i.e.* in Fig. 24 *a* and *b*). In larvae exceeding that length a second type of eye commences to develop, and is first noticeable in the form of a few small rounded pigmented patches situated above, and slightly behind, the larval eyes. These pigmented areas increase in number with the growth of the larva, and the newly added elements can be distinguished

by their nut-brown colour, whereas the already existing ones, owing to their greater amount of pigment, appear dense black (Fig. 24 *a*). These are the rudiments of the future imaginal compound eye ("Hauptauge"). As development proceeds, each of the small pigmented elements increases in size, with the result that it becomes in close contact with its neighbours, and in a larva about 7 mm. long they collectively form a conspicuous black crescentric shaped tract on either side of the head (Fig. 24 *b*). The subsequent extension of the organ is effected by new elements being added around its periphery.

A short optic nerve is present on either side in relation with the larval eye; in larvae about 7 mm. long a nerve from the imaginal ocular rudiment is distinguishable, and the two nerves of a side combine to form the main optic nerve (*b*. and *c*.).

When viewed in sections, the larval eye consists of a central densely pigmented area bordered by elongated cells whose narrower inner ends are directed towards the centre of the organ (*c*.). The eye is densely loaded with pigment of a rust-brown colour which appears black when viewed in thick layers.

The developing imaginal eye consists of a number of deeply pigmented fusiform bodies, placed at right angles with the surface of the head. These elements are the ommatidia of the compound eye, and they each consist, as seen in transverse section, of a central axis or rhabdom surrounded by a circlet of densely pigmented cells (the retinulae). Intervening between the ommatidia and the external cuticle is a layer of but little modified hypodermal cells (*h*). It is destined to form on the outside the corneal facets of the imaginal eye, and on its inside the crystal cells.

The cells of the hypodermis immediately bordering on the imaginal ocular rudiment are markedly columnar in form, and in places it is folded or invaginated into small pit-like areas, which are the very early stages in the development of new ommatidia.

In addition to the Culicidae, certain other families of nemocerous Diptera, having eucephalous larvae, are remarkable in that the imaginal eye develops in close relation with that of the larva and at an early stage in the larval life-history. A short comparative study of the eyes, in both the larvae and pupae of such Diptera, has recently been made by Zavřel (1907).

*General Remarks on the Larvae of the Culicidae.*

The larvae of the Culicidae may be defined as being aquatic, eucephalous, and with the first three post-cephalic segments fused together to form an evident and greatly enlarged thoracic mass. Although there is but little difficulty in identifying typical Culicid larvae, the greatly enlarged thorax affords the most evident *constant* morphological character, which is common to all the larvae of the family, and by means of which they may be readily separated from those of the allied Nemocerous families Dixidae, Psychodidae, Tipulidae and Chironomidae. It is true that, in the latter family, the first three post-cephalic segments become greatly swollen towards the end of larval life, in consequence of the developing imaginal organs contained within them. In the larval Chironomidae, however, there is no fusion into a compact thorax though the limits between the second and third segments may become partially obliterated. That the thorax of the Culicid larvae is composed of the first three post-cephalic segments is evident from the fact that it contains three of the ventral nerve ganglia, together with three double series of buds of the imaginal thoracic appendages, and moreover its musculature retains to some extent a trisegmental arrangement; externally, however, its composition is not by any means obvious. In *Corethrella brakeleyi* Coq. according to Johannsen (p. 401, pl. 40), the three segments can be perfectly clearly made out, since they have not undergone the same degree of fusion seen among other Culicid larvae. As a rule, the only external indication is to be seen in the principal hairs being arranged in three main transverse series. In all Culicid larvae the abdomen consists of nine segments.

Three principal types of modification are to be seen among Culicid larvae, viz.

(1) The *Anopheles* type where the tracheal system communicates with the exterior by means of a pair of spiracles situated on the dorsal aspect of the eighth abdominal segment, and the respiratory siphon is not developed. Palmate hairs are present on a variable number of the abdominal segments<sup>1</sup>, and the comb scales are replaced on either side by a chitinous plate bearing a series of posterior teeth.

<sup>1</sup> In some species (*A. fluviatilis* and *A. culicifacies*) a pair are present on the thorax and on each of the abdominal segments except the last (James, p. 25).

(2) The *Culex* type, in which the spiracles are situated at the extremity of a siphon formed as a drawing out or prolongation of the dorsal region of the eighth abdominal segment. The two main longitudinal tracheal trunks are usually of a much greater diameter than in the *Anopheles* type, and apparently function to some extent as "air reservoirs." There are no palmate hairs, and the comb almost always consists of a variable number of scale-like spines.

(3) The *Sayomyia* type is, on the whole, the most modified among the Culicidae. The spiracles and respiratory tube are absent and the tracheal system is greatly reduced and mainly consists of two pairs of air vesicles which are situated respectively in the thorax and seventh abdominal segment.

Theobald (1905) recognises eight sub-families among the Culicidae viz. the Anophelinae, Megarhininae, Toxorhynchitinae, Culicinae, Joblotinae, Aedomyinae, Heptaphlebomyinae and Corethrinae.

The *Anophelinae* form a tolerably natural group and exhibit a great similarity among their larvae. The most obvious differences that are to be found are seen in the characters of the frontal, plumose, and palmate hairs, the antennae, the form of the comb and the labial plate.

The *Megarhininae* comprise the single genus *Megarhinus*. The larva is of the *Culex* type, but is remarkable in having the comb represented by a large chitinous plate and in the pecten being absent. It is said to possess a rudimentary spiracle on the last abdominal segment (Felt, 1905, p. 445). The larva of *Toxorhynchites* very closely resembles that of *Megarhinus* and it has been pointed out by Christophers (1906, p. 13) that they agree very closely in the shape of the head and clypeus, in having the brushes modified into "clasping organs" for seizing their prey and in the anal gills being reduced to stump-like papillae.

In *T. immisericors* (Walk.) according to Theobald (vol. iii. p. 118) and Green (1905, p. 159) the siphon is excessively short, and there are no anal gills and the larva rests very nearly parallel to the surface film.

The *Culicinae* exhibit a wide range of variation in their larval structure. The most striking and variable organ is the siphon which differs greatly in its length and general shape among various genera. Stephens and Christophers obtain what they term the "siphonic index" by dividing the length of the tube by its maximum breadth. This index is useful for comparative purposes and appears, on the whole, to

be very constant in certain genera. According to Christophers in *Stegomyia* the siphonic index varies between 1·6 and 2; in *Desvoidaea* it is 1·7; in *Culex*, however, it varies very greatly being 4·3 in *Culex fatigans* Wied., while in *C. mimeticus* Noe, according to Theobald's figure (1903, vol. iii. p. 157) it is at least 14·5 on account of its extremely narrow diameter; in *Theobaldia* it is 6, while in *Taeniorhynchus* it is 13. In addition to the characters afforded by the siphon, numerous other structural variations occur, and some of which are proving of great utility in the specific determinations of the various larvae. The general form of the head, the characters of the antennae and the position of insertion of the antennal tuft, the form of the mouth-parts and especially of the labial plate are all of utility in the identification of species. Less useful characters are afforded by the variations in colour (very rarely) and in the form and arrangement of the cephalic and body setae. The anal gills present some degree of variation, being exceptionally long, according to Christophers, in a species of *Stegomyia* which mainly frequents the bottom of the water; in *Desvoidaea* they are large and broad (Theobald, vol. iii. pl. XVI), and in most of the known larvae of this sub-family they are well developed, though in *Culex tigripes* Grand. et Charm. and *Acartomyia*, according to Theobald's figures, they appear to be wanting. A wide variation is exhibited in the pecten, or rows of teeth situated on the siphon and, similarly, much variability is seen in the group of scale-like structures which are collectively termed the comb, and situated on either side of the eighth abdominal segment.

Perhaps, the most divergent larvae of the genus *Culex* are those of *C. mimeticus* which, in addition to the features of the siphon already mentioned, are remarkable on account of the large curved pecten spines (Christophers, p. 10), and the cannibalistic species *C. concolor* Robin. Des. and *C. tigripes*. Both of the latter are highly modified, showing but little relationship with the *Culex* larvae and whose affinities are with *Megarhinus* and *Toxorhynchites*. Christophers suggests provisionally the enlargement of the sub-family Megarhininae so as to include, in addition to *Megarhinus*, *Toxorhynchites* (thus doing away with the sub-family this genus represents), *Mucidus*, *Psorophora* together with *Culex concolor* and *C. tigripes*, and possibly *Janthinosoma* and *Lutzia*. All these forms are specially modified for cannibalistic and carnivorous habits in general. Their brushes are transformed into clasping organs for seizing their prey, the mouth-parts are also specially modified and the shape of the head and clypeus is very characteristic.



The anal gills are either reduced to mere stumps or are completely absent, and the attitude of these larvae in relation to the surface film is much more horizontal than is usual among larvae of the Culicinae. The two species, *C. concolor* and *C. tigrisipes*, Christophers proposes to place in a new genus *Jamesia*, and provided the larval characters are corroborated by sufficiently important differences in the imago, this procedure is fully warranted. Furthermore, the remarkable larva of *C. mimeticus* suggests the possibility that a new genus may be desirable in this instance also.

It seems highly probable that, as our knowledge of the Culicidae becomes more extended, a rational classification of the family will have to be based on a combination of both larval and imaginal characters, and in no other family of Diptera do the larvae apparently exhibit such exceptionally well-defined features, among both genera and species, for this purpose. At present however, until the morphology and ethology of the larvae have been more critically studied and how far any particular modification may be correlated with a certain mode of life, it seems very nearly impossible to discriminate between those characters which have been developed, perhaps recently, by adaptation and those which are to be regarded as phylogenetic or morphological characters. Until this distinction is clearly recognised the application of larval characters for the purposes of classification loses much of its value. Dyar and Knab (1906) have recently contributed an important paper in which they classify the larvae of the New World mosquitoes independently of the imagines. It remains to be seen, however, how far this scheme will fit in with any system of classification founded on imaginal characters.

Of the *Joblotinae* our knowledge of the larval stage is based on a very brief description given by Theobald (1903, vol. iii. p. 334) of the young larva of *Joblotia niveipes* and the later account of Goeldi (1905, p. 120). It appears to be an extremely divergent form with stout blunt antennae and a pair of remarkable "frontal processes." The latter, however, seem to be really the maxillae. There is a short respiratory siphon of very unusual form, but there seem to be no lateral comb.

In the *Aedomyiinae* the larval stages are very little known. In *Aedes fuscus* Osten-Sac. the larva is of the general *Culex* type and possesses four narrow lanceolate gills and a very short siphon (Dyar, 1902, p. 197). According to Felt (1904, p. 340) it so nearly resembles the larvae of *C. sylvestris* Thed. and *C. impiger* Walk. that it

is difficult to separate them. The larva of *A. smithii* Coq. differs, however, so greatly from that of *A. fuscus* that the systematist if he were relying solely on larval characters would unhesitatingly place it in a separate genus. According to Dyar (1901, p. 178) it possesses but a single pair of anal gills which are broad and rounded at their apices; in the number of its gills it seems to be unique among Culicid larvae and Dyar and Knab regard the upper pair as having been aborted. The mandibles have but a single bristle or curved spine at the apex and furthermore the pecten is entirely wanting. From *A. fuscus* it further differs in the form of the thorax and siphon.

In *Uranotaenia* the larva closely resembles that of a typical *Culex* but the comb is remarkable in that it consists of eight simple spine-like processes attached to the posterior margin of a lateral plate of chitin, and recalls the comb plate of the Anophelinae (Dyar, 1901, p. 179; Felt, 1904, p. 343). The larva of *Deinocerites* is only known from very brief notes given by Theobald (1903, vol. iii, p. 280) and it is not possible to compare it with other forms. His account is also rather obscure, since he confuses the thorax partly with the first two abdominal segments.

The *Heptaphlebomyinae* include but a single genus whose larva is unknown.

In the *Corethrinae* the larval stages are known for all the described genera. That of *Sayomyia*<sup>1</sup> represents the extreme type of larval development met with in this sub-family. It is almost perfectly transparent, the extremities of the jaws and the pigmented eyes and air reservoirs being the only parts that catch the unaided eye. The fore part of the head is much prolonged, carrying the antennae at the extremity. The pharynx is modified into an eversible tube, and there are remarkable posterior hooks carried on the last abdominal segment. Respiration appears to be almost entirely cutaneous, though a greatly reduced and much modified tracheal system is present. It consists of a pair of longitudinal trunks extending through the greater part of the animal, but does not contain air, except in the thorax and the seventh abdominal segment. In these positions, however, there are situated a pair of pigmented air reservoirs. There are no traces of spiracles present. Situated on the ninth segment of the abdomen are two pairs of anal gills.

The larva of *Corethra*<sup>2</sup> forms a connecting link between that of

<sup>1</sup> *Sayomyia* Coquill. (*Corethra* Loew).

<sup>2</sup> *Corethra* Meigen (*Mochlomyia* Loew).

*Sayomyia* and a typical Culicid larva. The intermediate condition is especially well indicated in the tracheal system which, although it has undergone some amount of reduction, is much better developed than in *Sayomyia* and, moreover, spiracles are present. They are situated at the extremity of a short respiratory siphon arising from the eighth abdominal segment. *Corethra* resembles *Sayomyia* in the form of the antennae but the head is not prolonged anteriorly (Meinert, Tab. II). The larva of *C. Karnerensis* is remarkable in having the extremity of the last segment bordered by a whorl of numerous, short, recurved fleshy processes (Felt, 1904, p. 353).

The larvae of both *Eucorethra* and *Corethrella* resemble *Corethra* much more closely than *Sayomyia*. Both possess a well developed tracheal system resembling that of *Culex* and opening at the extremity of a short siphon. The antennae in *Eucorethra* resemble those of the two preceding genera while those of *Corethrella* are curiously folded back against the head (Felt).

*Pelorempis* is a recently discovered form and very imperfectly known. It resembles *Sayomyia* and *Corethra* in the elongated antennae which are provided with stout spines set at an angle with their long axes. It has the general shape of the head of *Corethra* but the maxillae rather resemble those of *Sayomyia*. In its respiratory system it comes closer to the Anophelinae than to any other of the Culicidae. There is no siphon and the spiracles are supported by a chitinous skeleton resembling very closely that of *Anopheles* (Johannsen, p. 404). *Pelorempis* seems, therefore, to be a synthetic type as regards its larval characters, since it exhibits features belonging to both Corethrinae and Anophelinae.

The relationships of the larvae of the Corethrinae appear on the whole to come nearest to the Culicinae. The fact that they are predaceous, feeding on larvae of their own and other species, and small Entomostraca, &c., might suggest the possibility of their having arisen from the Megarhininae (*sensu latiore* of Christophers) which are similarly carnivorous (and cannibalistic). Such a view, however, would not be tenable since, in the Corethrinae, the antennae are the organs which are specially modified for seizing the prey, while in the latter group, it is the feeding brushes that have become modified for the same purpose.

Rondani in 1856 appears to have been the earliest writer to separate the Corethrinae from the rest of the Culicidae and elevate them to family rank. More recently Coquillett and others have also

advocated this classification. Great stress has been laid on the reduced condition of the mouth-parts which are not adapted for piercing and, correlated with it, the feeding habits of the female. Additional characters have also been found in the neuration of the wings and the absence of scales from the latter. It is the belief of the author of this paper, however, that the Corethrinae are best retained as a sub-family of the Culicidae.

The reasons for this conclusion are as follows:—

The newly discovered genus *Pelorempis*, though agreeing with *Sayomyia* and *Corethra* in the form of the proboscis, resembles the Culicinae in possessing scales to the wings. The larva, moreover, as has already been pointed out, greatly differs from those of the remaining Corethrinae. The *Sayomyia* larva, it is true, is totally different from any other Culicid larva, but those of the remaining Corethrinae are clearly intermediate in the structure between that genus and the general *Culex* type of larva. It is, therefore, clear that the limits of the Corethrinae cannot at present be very well defined and, for that reason, it is better to retain the group as a sub-family only.

The resemblances between the larvae of the Anophelinae and the Culicinae, as regards their internal anatomy (the presence of cardiac coeca and five Malpighian tubes), and the great divergence between those groups and the genus *Sayomyia* (four Malpighian tubes and the absence of cardiac coeca), are sufficient to suggest that an anatomical study, of typical representatives of the different groups, may prove a valuable aid towards a rational classification of the family.

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## REFERENCE LETTERING.

abd. 5, 6, 8, 9.	Fifth, sixth, eighth and ninth abdominal segments.
abd. g. 1.	First abdominal nerve ganglion.
abd. g. 8 + 9.	Ganglion formed by the conrescence of those of the eight and ninth abdominal segments.
al. m.	Alary muscle.
a. m.	Adductor muscle of mandible.
a <sup>l</sup> . m <sup>l</sup> .	Abductor muscle of mandible.
a. n.	Antennal nerve.
ao.	Aorta.
ap.	Aperture of salivary duct.
at. d.	Imaginal bud of antenna.
at. m.	Antennal muscle.
b.	Brush.
b. m.	Inner retractor muscle of brush.
b <sup>l</sup> . m <sup>l</sup> .	Outer " " " "
c.	Cuticle.
c. c.	Lateral cellular cord.
c. com.	Cerebral commissure.
d.	Budiment of generative duct.
d. tr.	Main longitudinal tracheal trunk.
d. v.	Dorsal vessel.
e.	Eye.
ep. m.	Epipharyngeal muscles.
f. b.	Fat-body.
f. n.	Frontal nerve.
f. pl.	Fan-like plate.
g.	Abdominal nerve ganglion.
g. d.	Imaginal buds of gonapophyses.
g. l.	Ganglionic layer.

<i>h.</i>	Hypodermis.
<i>i. mus.</i>	Inner vertical muscle of spiracular lobe.
<i>l. d.</i>	Imaginal bud of left middle leg.
<i>l'. d¹.</i>	Imaginal bud of labium ; left of the paired elements (section somewhat oblique).
<i>l. e.</i>	Larval eye.
<i>l. musc.</i>	Longitudinal muscles of spiracular lobe.
<i>l. pl.</i>	Lateral plate of spiracular lobe.
<i>l. t. m.</i>	Longitudinal tergal muscles.
<i>m.</i>	Muscle attached to supporting collar of heart.
<i>m. b.</i>	Striated muscle bands (in section).
<i>m. c.</i>	Mother cells.
<i>m. g.</i>	Mid-gut.
<i>m. n.</i>	Median nerve.
<i>m. pl.</i>	Median plate of spiracular lobe.
<i>m. s.</i>	"Mesenchyme."
<i>m. x.</i>	Muscle of maxilla.
<i>ner.</i>	Lateral nerve.
<i>o.</i>	Ommatidia.
<i>oen.</i>	Oenocytes (smaller).
<i>o. mus.</i>	Outer vertical muscles of spiracular lobe.
<i>o. n.</i>	Optic nerve.
<i>p.</i>	Chitinous peg.
<i>p. c.</i>	Pericardial cells.
<i>ph.</i>	Pharynx.
<i>ph. w.</i>	Wall of pharynx.
<i>pl.</i>	Lateral toothed plate.
<i>p. m.</i>	Peritrophic membrane.
<i>p. s.</i>	Peripodial space.
<i>r.</i>	Rectum.
<i>sc.</i>	Basal supporting plate of spiracular skeleton.
<i>s. col.</i>	Supporting collar of heart ( <i>anneau de soutien</i> ).
<i>s. col. p.</i>	Posteriorly directed prolongations of the supporting collar.
<i>s. d.</i>	Median salivary duct.
<i>s. g.</i>	Sub-oesophageal ganglion.
<i>sp.</i>	Spiracles.
<i>sup. g.</i>	Brain.
<i>t.</i>	Tracheal trunk to anal gills of its side.
<i>t. b.</i>	Transverse chitinous band.
<i>t¹. b¹.</i>	Transverse intersegmental band.
<i>t. f.</i>	Larval "tail-fin."
<i>t. f. m.</i>	Muscles of larval "tail-fin."
<i>t¹. f¹.</i>	Terminal filament.
<i>t. f. d.</i>	Imaginal bud of pupal "tail-fin."
<i>th. g. i.</i>	Prothoracic ganglion.
<i>t. pl.</i>	Terminal plate of spiracular lobe.
<i>tr.</i>	Trachea.
<i>w. d.</i>	Imaginal bud of wing.
<i>x - - y</i>	Direction of the transverse section Fig. 2.

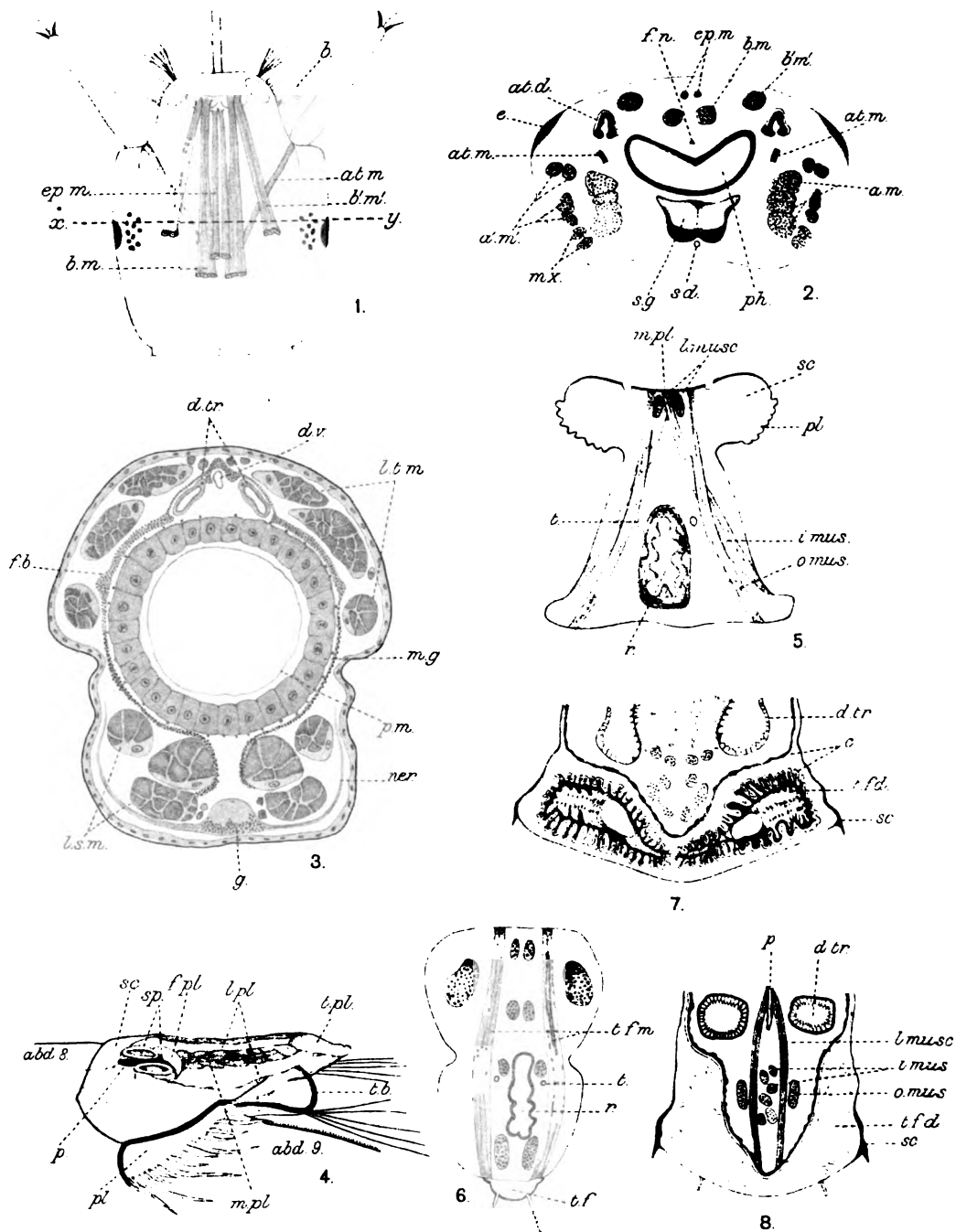
## DESCRIPTION OF THE PLATES.

## PLATE IX.

- Fig. 1. Dorsal aspect of the head of a larva of *Anopheles maculipennis* showing certain of the muscles.
- Fig. 2. Transverse section through the head across the line  $x-y$  in the preceding figure.
- Fig. 3. Typical transverse section through the abdomen and passing through one of the ventral nerve ganglia.
- Fig. 4. Lateral view of the spiracles and their supporting skeleton.
- Fig. 5. Transverse section through the eighth abdominal segment together with the spiracular lobe. (Reconstruction from several sections.)
- Fig. 6. Transverse section through the ninth abdominal segment showing the muscles of the "tail-fin." (Reconstruction from several sections.)
- Fig. 7. Longitudinal and horizontal section through the spiracular lobe passing some distance below the level of the spiracles.
- Fig. 8. Do. passing just beneath the spiracles.
- Fig. 9. Transverse section through the heart and associated structures; 6th abdominal segment.
- Fig. 10. Dorsal aspect of a portion of the heart showing alary muscles and pericardial cells; 6th abdominal segment.
- Fig. 11. Rudiment of the male gonad from larva measuring 6.5 mm. long.
- Fig. 12. Rudiment of the female gonad from a larva measuring 6.75 mm. long. The numbers in this and the preceding figure represent the respective segments in which the organ lies, and the horizontal lines indicate the boundaries between those segments.
- Fig. 13. Transverse sections through three separate regions of the male gonad of a larva about 6 mm. long: (a) through the base of the terminal filament; (b) through the middle of the gonad; (c) through the developing duct.
- Fig. 14. Outline figure of the rudiment of the male gonad in a larva 2.3 mm. in length.
- Fig. 15. Transverse section through the heart and associated cellular cords; anterior part of the thorax.
- Fig. 16. Do. through the heart and posterior prolongations of the supporting collar; commencement of oesophagus.
- Fig. 17. Do. through the middle of the supporting collar together with the heart.
- Fig. 18. Do. through the cerebral commissure and the anterior termination of the heart.
- Fig. 19. Section of imaginal buds of wing and second leg of left side at an early stage in development.
- Fig. 20. Section through imaginal antennal bud and passing through the opening of the primitive invagination.
- Fig. 21. Longitudinal and vertical section passing through the cerebral commissure together with the terminal portion of the heart and the associated supporting collar.

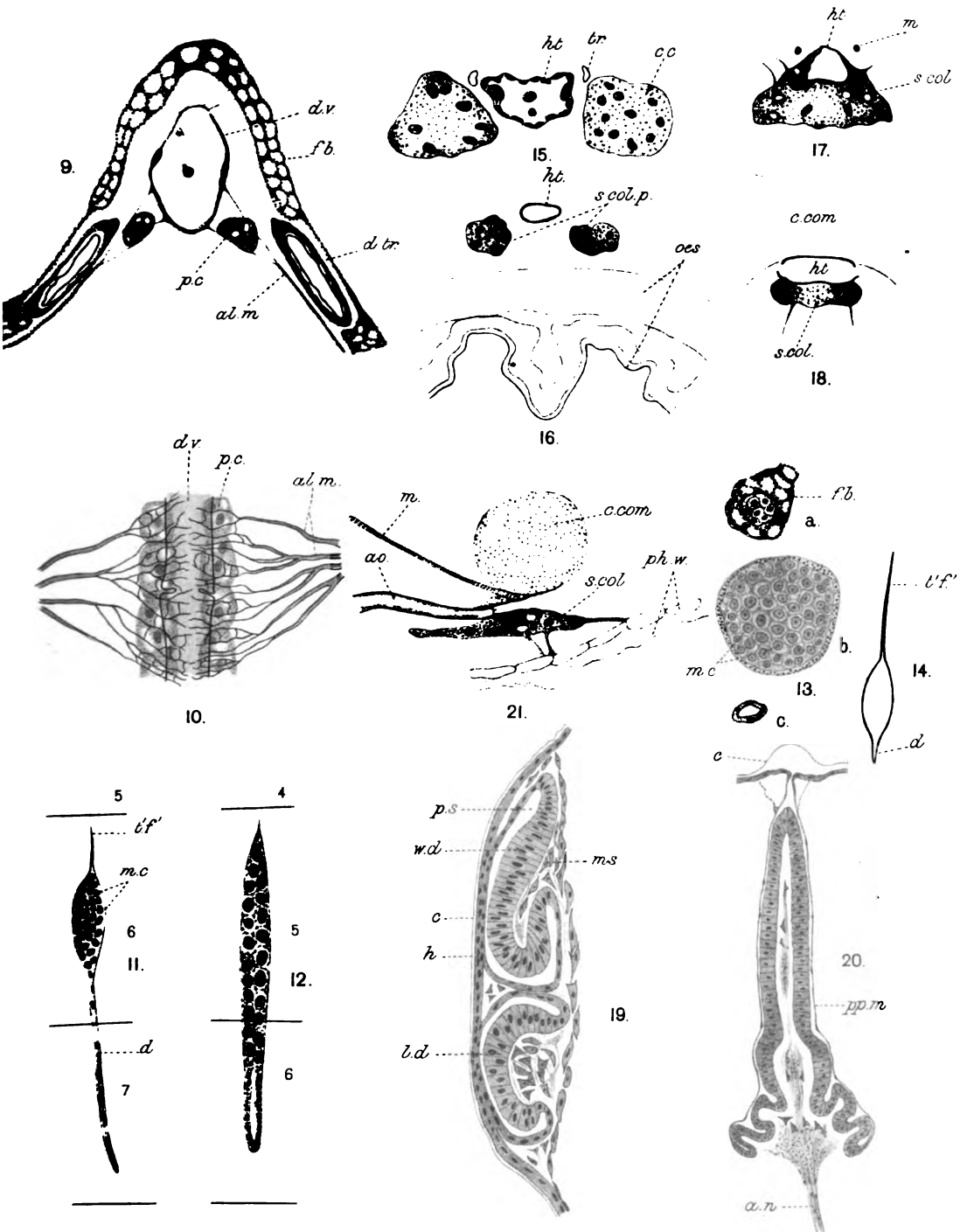






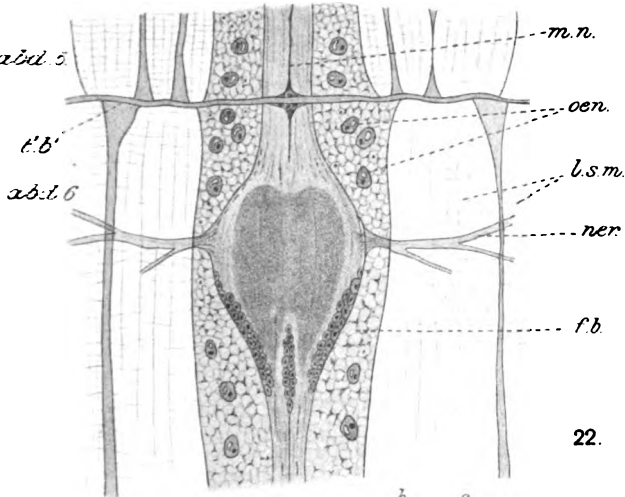
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PLATE IX

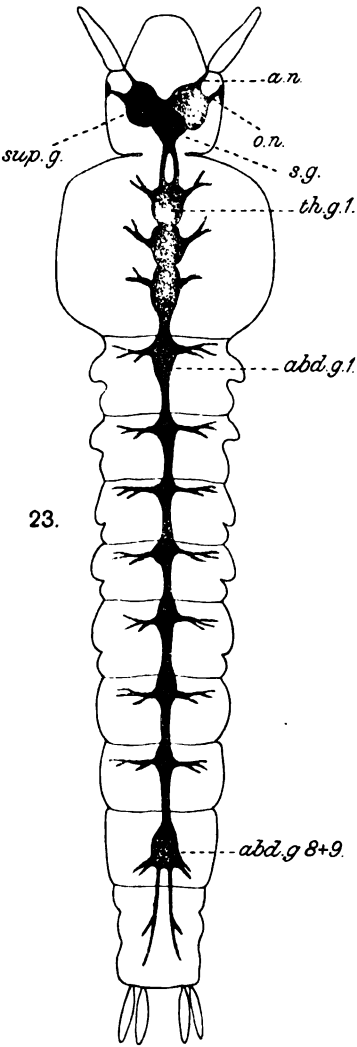


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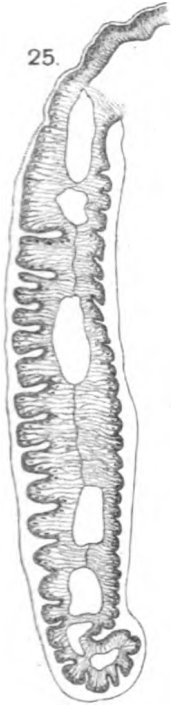




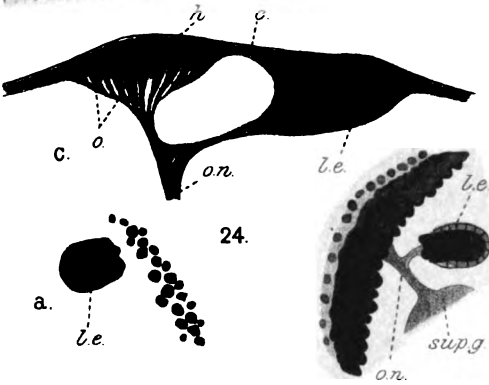
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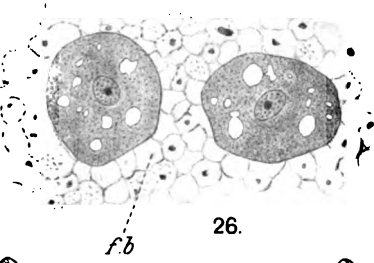
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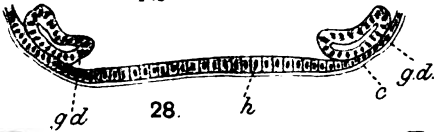
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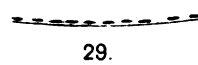
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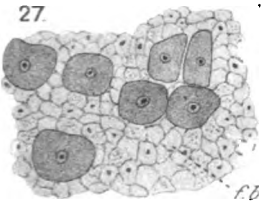
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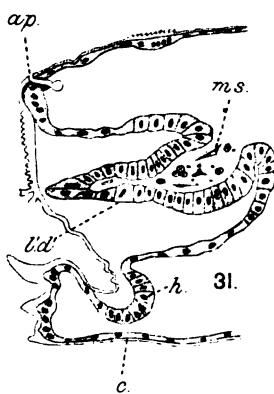
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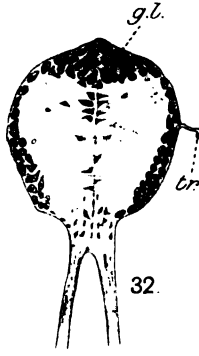
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31.



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E. Wilson, Cambridge



## PLATE X.

- Fig. 22. Figure of the ventral region of the 6th abdominal segment showing its nerve ganglion, the fat-body and oenocytes. Longitudinal and horizontal section.
- Fig. 23. Nervous system of a larva about half-grown.
- Fig. 24. Larval and developing imaginal eyes : (a) from a larva 4.25 mm. long ; (b) from a larva 7 mm. long ; (c) do. viewed in section. Figs. a and b are from larvae cleared and mounted whole in Canada balsam.
- Fig. 25. Section through left imaginal wing bud : fully grown larva.
- Fig. 26. A pair of the larger oenocytes : 5th abdominal segment.
- Fig. 27. Group of the smaller oenocytes.
- Fig. 28. Section through ventral integument and imaginal buds of gonapophyses.
- Fig. 29. Longitudinal section through the walls of the heart : 8th abdominal segment.
- Fig. 30. Portion of the heart showing striated fibres : 8th abdominal segment.
- Fig. 31. Longitudinal and vertical section through labial plate and imaginal labial bud (left rudiment).
- Fig. 32. Longitudinal and horizontal section through the sub-oesophageal ganglion.

THE MODE OF MULTIPLICATION OF *PIROPLASMA BOVIS*,  
*P. PITHECI* IN THE CIRCULATING BLOOD COMPARED  
 WITH THAT OF *P. CANIS*, WITH NOTES ON OTHER  
 SPECIES OF *PIROPLASMA*.

Plate XI and Diagrams I—IV.

By GEORGE H. F. NUTTALL, M.D., Ph.D., Sc.D., F.R.S.  
*Fellow of Magdalene College, Quick Professor of Biology in the University  
 of Cambridge.*

AND G. S. GRAHAM-SMITH, M.A., M.D.  
*University Lecturer in Hygiene, Cambridge.*

IN our last paper dealing with *Piroplasma canis* (*Journal of Hygiene*, April 1907, VII. pp. 232—272), we described the usual mode of multiplication in the circulating blood, as ascertained by a long series of observations on the *living parasite*. In summarising these observations we say (p. 250):—

“*Piroplasma canis* has a free and an intracorpuseular stage in the blood of the dog, and it is during the latter stage that multiplication occurs. This asexual multiplication takes place in one of the following ways.

(1) A free pyriform parasite which has just left a blood corpuscle enters a normal corpuscle and assumes a round form, remaining quiescent for a time. The round body then grows and, after passing through an actively amoeboid stage, again becomes rounded. Two symmetrical processes are then protruded, which rapidly enlarge at the expense of the body of the parasite. Each of these processes gives rise to a mature pyriform parasite, which remains for a time joined to its fellow by a thin strand of protoplasm. On the rupture of the containing corpuscle these pyriform parasites become free and enter other corpuscles.



(2) Occasionally by the protrusion of four processes four mature pyriform parasites are formed from a single amoeboid form.

(3) Sometimes a young rounded intracorpuseular parasite divides by simple division and gives rise to two amoeboid parasites, which grow and divide by the protrusion of symmetrical processes each into two pyriform parasites, thus giving rise to four mature parasites within the corpuscle. Sometimes the two amoeboid parasites undergo the processes of division simultaneously, but not infrequently one is considerably in advance of the other.

(4) It is possible that occasionally a red blood corpuscle is invaded either simultaneously or at different times by two pyriform parasites, each of which undergoes the changes described above.

(5) Several pyriform bodies within a single corpuscle are produced by the division in the manner described of one or more amoeboid parasites.

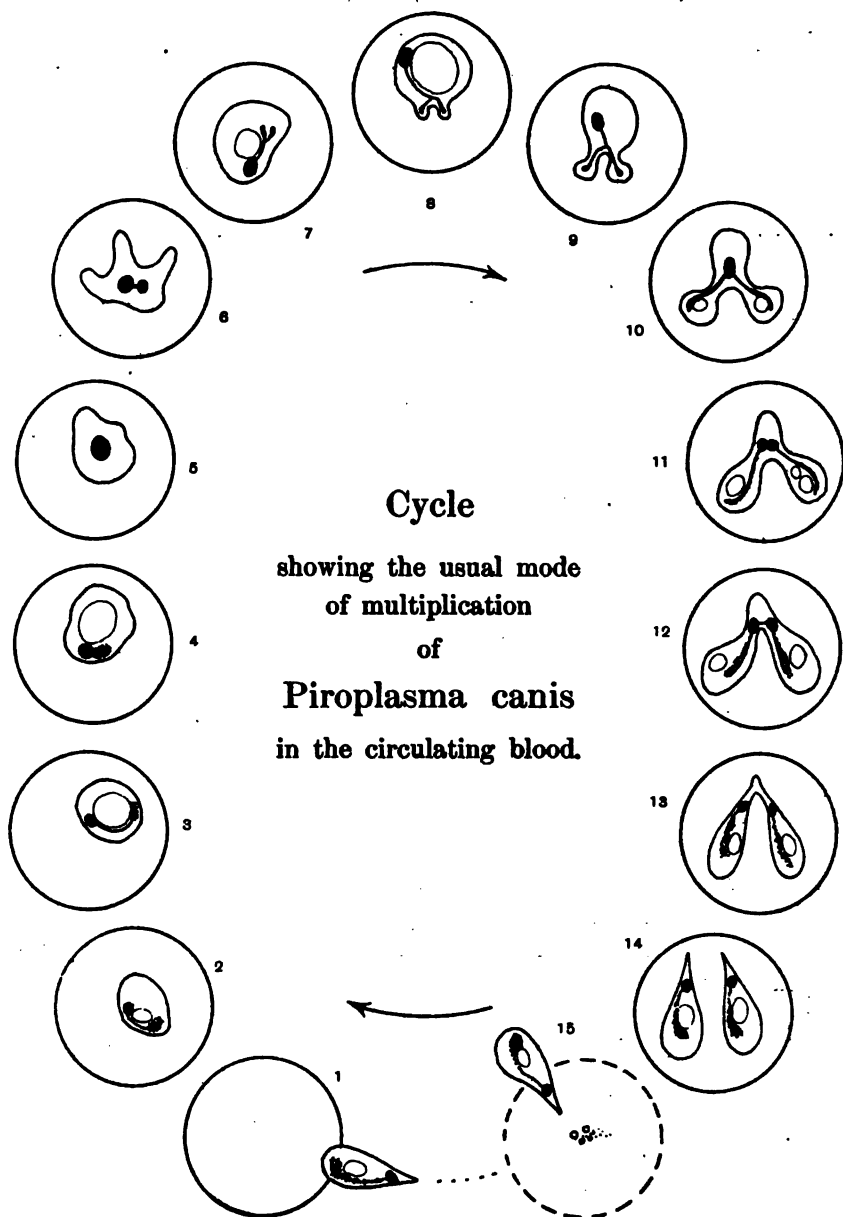
All parasites which have not reached the mature stage, when the containing corpuscle ruptures, rapidly degenerate and die in the plasma. The same is true of mature pyriform parasites, which do not, after becoming free, soon enter other corpuscles."

Aided by the knowledge thus gained we were able, by the examination of stained films, to figure the nuclear (chromatin) changes which accompany each phase of division (1907, Plate II). A schematic representation of the mode of multiplication was given on p. 258, and is reproduced in this paper (p. 136). We now figure some of the more important stages by means of photomicrographs (see Plate XI).

Christophers (1907, p. 17), who at the time of writing had apparently not seen the paper just quoted, and who seems to have formed his conclusions on the study of stained specimens only, thinks that besides the method of division we have described the formation of the pear-shaped forms by fission occurs in two other distinct ways.

"(1) In the case of certain large parasites, characteristic of early invasion and infection in young dogs, the division takes place in the manner described by Nocard and Motas. After the chromatin has divided a dark line forms across the parasite in the centre of which a cleft is later seen. This stage gives rise to two closely approximated bodies. (2) A method of division, almost if not quite as common, takes place when a parasite is stretched across a cell. The long oval becomes constricted in the centre, and the two ends passing away from one another become two pear-shaped bodies."

Although we have made very prolonged and careful studies at all



*Diagram I.* (Reprinted from *Journ. of Hygiene*, vol. VII. p. 258.)

stages of infection by inoculation on the large and elongated forms seen in the living blood we have never observed the formation of pyriform bodies by either of these methods, and cannot accept conclusions based

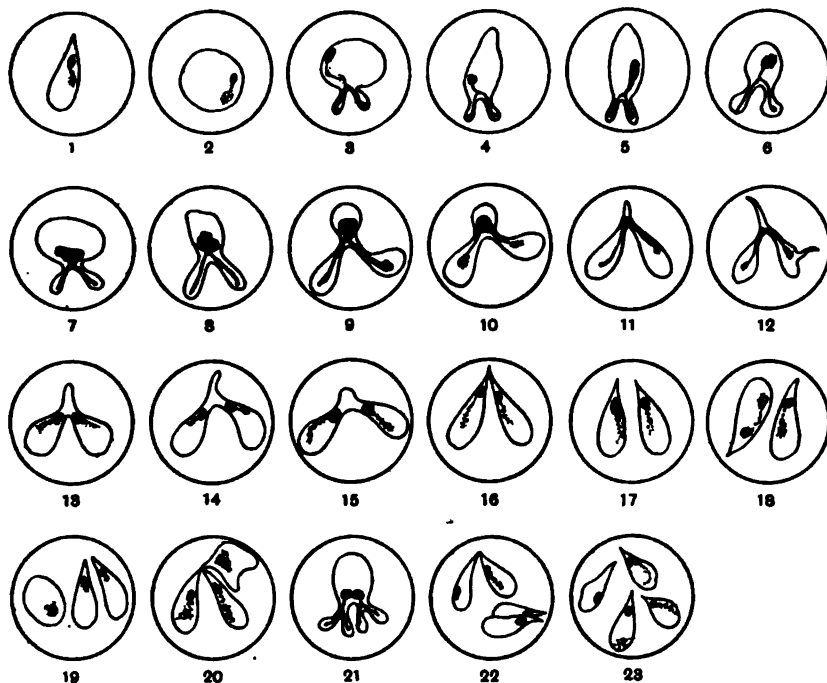


Diagram II. (*Piroplasma bovis*.)

Explanation of Diagram II showing multiplication forms of *P. bovis* compared with those of *P. canis* (Diagram I).

Stages figured in Diagram II ( <i>P. bovis</i> )	...	which correspond to	...	Stages figured in Diagram I ( <i>P. canis</i> )
Figs. 1, 2, 3, 4, 5, 6	...	...	...	1, 2, 8, 8, 9, 9.
„ 7, 8, 9	...	...	...	Stages between 9 and 10.
„ 10, 11	...	...	...	10.
„ 12	...	...	...	11.
„ 13, 14, 15	...	...	...	Stages between 12 and 13.
„ 16	...	...	...	13.
„ 17, 18	...	...	...	14.

The remaining stages correspond to other Diagrams as follows:

Figs. 19, 20	...	...	1906, Diagram 6, Figs. 1, 2 (p. 596).
„ 21	...	...	1907, Diagram 27, Fig. 3 (p. 240).
„ 22, 23	...	...	1906, Diagram 6, Figs. 7-16.

on the study of stained specimens against the evidence derived from observations on living parasites.

In the paper quoted we have shown that single parasites do undoubtedly divide by simple division (Diagram 28, p. 240) but in this case give rise to two amoeboid forms, each of which may proceed to the formation of pyriform bodies (Diagrams 30, 31 and 36, pp. 241, 242, 262) in the manner we have described.

Stained specimens of such preliminary divisions and apparent divisions (Diagram 29, p. 241) may have influenced Christophers in his conclusions.

In stained preparations *Piroplasma canis* can be distinguished from other intracorpuseular blood parasites by the occurrence of intracorpuseular pyriform bodies, which represent the mature form of the parasite in the blood. These pyriform bodies are most commonly present in the corpuscles in pairs, though multiples of two, four, eight and sixteen, are not uncommon. These forms generally show two chromatin masses, a dense and compact mass usually situated near the pointed end and a secondary mass of loose chromatin<sup>1</sup> which may be arranged in various ways, extending towards the blunt end.

Dividing forms, fixed in the later stages of division, especially the trilobed forms which are of frequent occurrence in suitable films, are yet more characteristic. By a careful study of stained films we have been able to establish the fact that two other species of *Piroplasma*, *P. bovis* and *P. pitheci*, divide in the same manner as *P. canis*, and show the same chromatin masses.

#### *Piroplasma bovis.*

After examining a considerable number of blood films, each of which contained only a few parasites with negative results as regards the finding of dividing forms, we obtained a slide prepared from a cow suffering from a high degree of infection; through the kindness of Mr J. R. Jackson, M.R.C.V.S., of the Board of Agriculture and Fisheries. In parts of this film as many as 30 infected corpuscles could be counted in a single field. Double but especially single pyriform bodies containing both dense and loose chromatin were common. The film was

<sup>1</sup> This secondary mass of chromatin was first described by us (*Journal of Hygiene*, x. 1906, p. 590) and has since been noticed by Christophers (1907, p. 22) in *P. canis*, who says "the loosely packed area described by Nuttall and Graham-Smith is almost invariably present," and by Fantham (1907, p. 308) in *P. bovis*.

taken at a period of very active multiplication, for typical dividing forms were found in almost every field<sup>1</sup>.

We have figured a selected series in Diagram II, which may be compared with our schematic figure of the usual mode of multiplication in *P. canis* which we have reproduced (Diagram I).

*P. pitheci*, P. H. Ross, 1905.

This parasite was discovered by Dr Philip H. Ross in the blood and internal organs of a species of *Cercopithecus*, and was described by him in the *Journal of Hygiene*, I. 1905, v. pp. 18—23. As he did not illustrate the parasite the true nature of the organism still remained in doubt.

Through the kindness of Dr Ross we have had the opportunity of studying his slides. One blood film contained only a single parasite, but another contained over one hundred. A smear preparation from the liver contained large numbers of parasites but was not as well fixed or stained as the blood preparations. All these preparations were systematically examined and the parasites sketched<sup>2</sup>.

These observations have convinced us that the parasite described by Ross is a true *Piroplasma*, and multiplies in the same manner as *P. canis* and *P. bovis*.

The number of parasites contained in infected corpuscles varied from 1 to 16.

Number of parasites per infected corpuscle		
	In blood films	In liver smear
1	Common	Common.
2	"	"
3	One found	None found.
4	Found 7 times	Fairly common.
5	Not found	Not found.
6	"	"
8	Found 6 times	Fairly common.
16	Not found	Two found.

The prevalence of the paired forms and the occurrence of the usual multiples 2, 4, 8, 16, accord with what we have observed in *P. canis*.

<sup>1</sup> Of 500 consecutive infected corpuscles examined 55% contained single parasites, 34% double parasites and 11% contained parasites in the typical process of division. We would particularly emphasize the fact that numerous dividing forms are only to be occasionally met with, consequently negative results, on the examination of single films, or even a series of films from a single animal, cannot be accepted as evidence against this being the usual mode of division.

<sup>2</sup> Our thanks are due to Mr C. Strickland, who searched through the second blood preparation with great care.

The series of figures included in Diagrams III and IV are selected from the large number of sketches which we made.

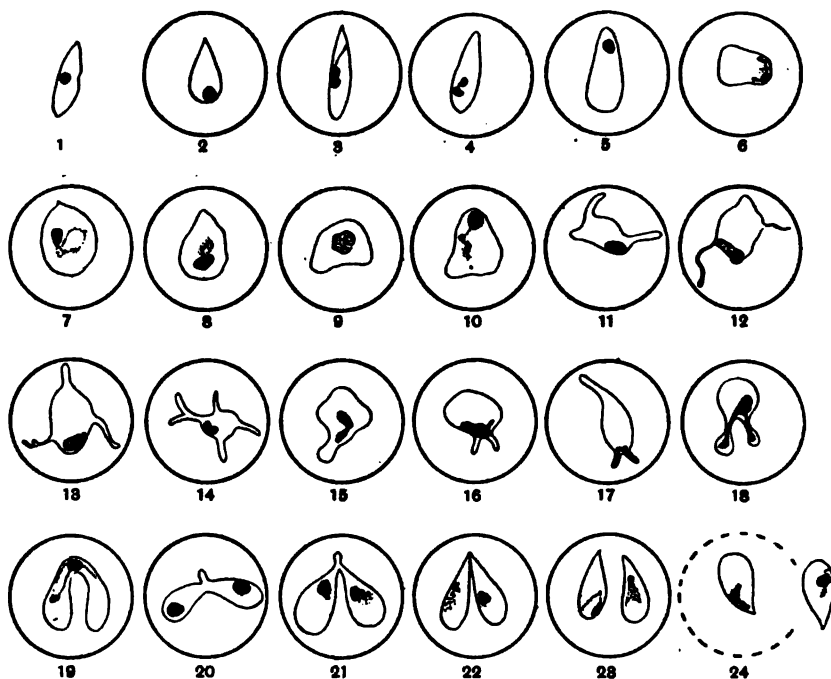


Diagram III. (*Piroplasma pitheci*.)

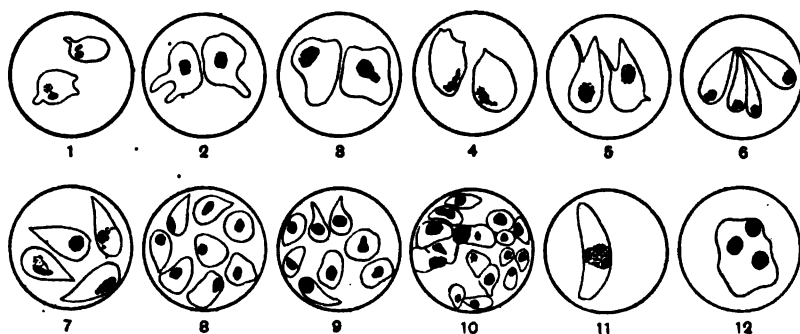


Diagram IV. (*Piroplasma pitheci*.)

We have described and figured similar stages in *P. canis* (see Diagram I, p. 136; also *Journal of Hygiene*, x. 1906, and iv. 1907.

*Piroplasma muris*, Fantham, 1906.

Through the courtesy of Mr H. B. Fantham we have been able to examine one of his films prepared from the blood of a rat infected with *P. muris*. This preparation contained pyriform intracorpuseular parasites, singly and in pairs. In the plate accompanying his paper corpuscles infected with four and six parasites are shown. The secondary mass of loose chromatin has not been observed and no typical dividing forms have been found. The latter fact may partially be accounted for by the low degree of infection and the lack of material. Further the fact that the disease is a chronic one renders the presence of many dividing forms at one time improbable.

*Piroplasma equi* and *P. ovis*.

We have as yet been unable to secure satisfactory specimens of these organisms, and none of the papers which have been published have contained figures or records of dividing forms such as we have described in *P. canis*.

Note on *Achromaticus vesperuginis*, Dionisi 1898.

This parasite is regarded as a *Piroplasma* by Sambon<sup>1</sup>, 1908, in Manson's *Tropical Diseases* (4th edition, p. 841), but we fail to see sufficient reason for this assumption. It is true that both Dionisi and Gonder figure a few pyriform parasites amongst the many they illustrate, and we have found similar forms in films Dionisi gave to one of us in 1899.

In only one other respect does this organism resemble a typical *Piroplasma*, namely in the occurrence of four pyriform parasites within the corpuscle. Further investigations are required before its position can be settled<sup>2</sup>.

## SUMMARY.

In stained preparations *Piroplasma canis*, *P. bovis* and *P. pitheci* may be distinguished from other intracorpuseular parasites by the presence of intracorpuseular pyriform bodies, usually occurring in pairs and less commonly in fours, eights and sixteens. These pyriform bodies show a dense mass of chromatin near the pointed end and a loose mass, often connected with the dense mass, situated towards the blunt end. In suitable preparations peculiar dividing forms, most typically represented by trilobed forms or more or less pyriform bodies joined to a single smaller rounded or elongated mass of protoplasm, may be seen.

<sup>1</sup> We regret to note several inaccurate statements which occur in Sambon's section dealing with the genus *Piroplasma*.

<sup>2</sup> Gonder (1906, pl. IV) retains the name *Achromaticus* believing it to be intermediate between human malarial parasites and *Piroplasma*.

In the absence of observations on the *living parasite* we consider that these points may be taken as characteristic of the genus *Piroplasma*.

In spite of the fact that dividing forms have not yet been found and that the secondary mass of chromatin has not yet been observed *P. muris* may perhaps be included in this genus.

*Piroplasma quadrigemium*, an intracorpuseular parasite recently observed by Nicolle (XI. 1907) in a small North African rodent (*Ctenodactylus gundi*), apparently divides in a totally different manner, and shows a peculiar disposition of the chromatin. No loose chromatin has been observed. Until further observations have been made this parasite cannot be included among the true *Piroplasma*.

Further observations are needed before the position of the other so-called *Piroplasma* can be determined.

The expenditure entailed in the prosecution of our investigations on piroplasmosis has been largely defrayed by grants from the Government Grants Committee of the Royal Society.

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#### EXPLANATION OF PLATE XI.

Multiplication of *P. canis*. Examples taken from a single blood-film. Photomicrographs,  $\times 8000$ .

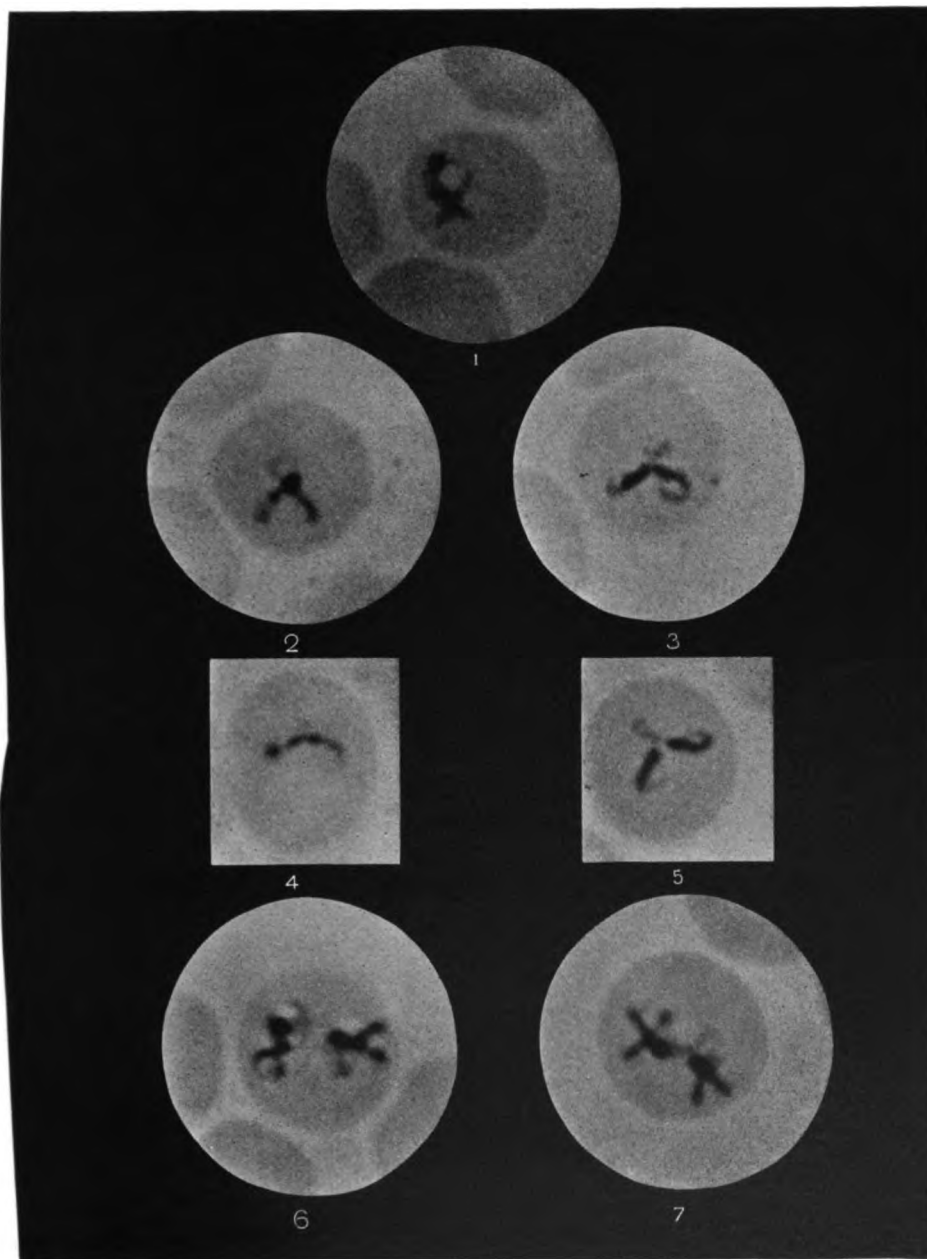
Figs. 1, 2 correspond to Figs. 8—10 in Diagram I (p. 136).

Figs. 3, 4 correspond to Fig. 12.

Fig. 5 corresponds to Fig. 18.

Figs. 6, 7 correspond to Figs. 7, 8 in Diagram 36 (1907, p. 262), and each illustrate the simultaneous division of two parasites in a corpuscle.





**PIROPLASMA CANIS**  
Illustrating the process of multiplication.



## NOTE ON THE BEHAVIOUR OF *SPIROCHAETAE* IN *ACANTHIA LECTULARIA*.

By GEORGE H. F. NUTTALL, F.R.S.

*Fellow of Magdalene College, Quick Professor of Biology in the  
University of Cambridge.*

A POPULAR belief prevails in Russia that the bug *Acanthia lectularia* plays a part in the spread of relapsing fever. Flügge (1891) however appears to have been the first scientific writer to suppose that vermin might spread the disease. This view was also held by Tictin (1897) who considered that man may become infected by (a) being bitten by bugs which had previously fed on blood containing *Spirochaeta obermeieri*, or (b) by his crushing such bugs and infecting himself with the spirochaetes through lesions in the skin induced by scratching. Tictin injected the gut-contents of infected bugs which had recently fed on a relapsing fever patient into monkeys and infected them. When the bugs were crushed 48 hours after feeding on infected blood their contents did not produce the disease in monkeys.

Karlinski (1902) observed *Spirochaeta obermeieri* in 120 bugs examined during the prevalence of relapsing fever in Bosnia. The bugs were collected in houses where cases of relapsing fever occurred. The spirochaetes retained their motility in the bugs for 30 days after the initial feeding, but later they appeared to be degenerating. (The examination of fleas and lice gave negative results.) Schaudinn, not long before his death, informed me that he had observed an even longer persistence of the spirochaetes in bugs. Christy (1902), working in Bombay, placed bugs in the beds of relapsing fever patients and afterwards allowed twelve of the bugs to bite him, one bug biting him each day on twelve successive days. He did not however acquire relapsing fever. Breinl, Kinghorn and Todd (1906, p. 113) do not state that they made

observations on the behaviour of spirochaetae in bugs. They however published the results of ten experiments (conducted in Liverpool) on monkeys which they sought to infect with spirochaetes through the agency of *Acanthia lectularia*. Six of the ten experiments were carried out with *Spirochaeta duttoni* alone, whilst four were carried out with bugs infected partly with *Sp. obermeieri* (American strain) and partly with *Sp. duttoni*. It is not quite clear from their paper how many bugs were infected with the latter spirochaete alone. Considering the experiments collectively, the authors named allowed 10 monkeys to be bitten by 2210 bugs which had previously fed on infected animals. The bugs sucked infected blood at different stages of the disease and in turn bit fresh uninfected monkeys at variable periods of time (2 to 3 or 10 to 15 days) after being infected. A few of the bugs were kept at a low temperature (5.5°C.) after sucking infected blood, but the majority were maintained at 20—21°C. All their results were negative.

Klodnitzky (1908, p. 127) in Astrachan, allowed bugs to bite relapsing fever patients and also collected supposedly infected bugs in hospital wards. He examined the contents of about 30 bugs and detected isolated spirochaetes in them for 3—5 days after they had fed, and subsequently he observed what he supposed was a great multiplication of the spirochaetes in the bugs. His description and the two photomicrographs which illustrate his paper leave no doubt in my mind but that he took the spermatozoa of the bug for spirochaetes.

From the foregoing it will be seen that we still lack evidence proving that *Sp. obermeieri* is conveyed by bugs. The negative results obtained by Breinl, Kinghorn and Todd do not however exclude the possibility of bugs being the carriers of relapsing fever, and certainly the results obtained with *Sp. duttoni* do not weigh as evidence against bugs being the carriers of *Sp. obermeieri*. There is no evidence to support the hypothesis of Dönitz (1907, p. 18) that European relapsing fever is conveyed by ticks. The observations by Karlinski and Schaudinn above cited indicate that further work on the subject is still required.

Failing *Spirochaeta obermeieri* it appeared to me desirable to study the behaviour of *Sp. duttoni* in *Acanthia lectularia* and with this object the following experiments were carried out.

*Acanthia lectularia*, received from London, were placed at 16°C. Hungry specimens were chosen and fed on mice in whose blood *Sp. duttoni* was present in large numbers. At the time stated in the first column, in minutes or hours, a bug (or two) was dissected and the intestinal contents examined fresh and stained by Giemsa's method (dry films).

*Experiment I, at 12° C.*

Bugs fed on	Time when bug was examined after feeding	Appearances of spirochaetes in gut-contents	
		Fresh	Stained
Series A 30. iv. 1907	10, 15, 15 mins.	Many, very active	Rigid spirals, few wormlike spirochaetes, all stain well incl. red blood corpuscles.
	½, 1, 1½ hrs.	Ditto, some spir. sluggish	Ditto.
	21, 27 „	All fairly active	Ditto.
	48, 74½ „	Fairly active, some sluggish	Ditto, but some r. b. c. stain faintly.
	95 „	All fairly active	Ditto.
Series B 27. v. 1907	100, 117 „	Sluggish	Ditto.
	139 „	Saw only one moving: sluggish	Ditto.
	164, 190 „	Motionless	Ditto.
	210, 234 „	„	Ditto, corpuscles few and vanishing.
	254, 278 „	„	Ditto.

*Experiment II, at 14° C.*

3. v. 1907	132 hrs.	Very slight motion, spir. tangled	Many spirals, few wormlike, stain well; r. b. c. traces?
	144 „	Slight motion?	Ditto.
	167, 196, 218 hrs.	Motionless	No r. b. c. seen, spir. stain well.

*Experiment III, at 16° C.*

2. iv. 1907	10 mins.	Many, very active	Many, all wormlike.
	3½, 6½ hrs.	Fairly active	Few wormlike, mostly spirals.
	19 „	Active, in bunches	Mostly spirals, some thick, beaded or irregular; r. b. c. stain well.
	28 „	Fairly active, some motionless	Ditto, many spir. appear thick.
	42 „	Slight motion	Spirals stain faintly, r. b. c. vanishing.
	72 „	Not examined	No. spir. found, no r. b. c.
	92, 117 „	„ „	Fairly numerous spir., stain well.
	139 „	Few, motionless	Ditto, but stain faintly.

*Experiment IV, at 20° C.*

2. iv. 1907	8½ hrs.	Fairly active	Spirals and r. b. c. both normal.
	6 „	Fairly active, some motionless	Spirals, some beaded and irregular, r. b. c. vanishing.
	8½ „	Motionless	Spirals, dividing forms, beaded and irregular forms, very few r. b. c.
	22½, 31 „	„	Mostly faint-staining, often fragmented, few or no r. b. c.
	46, 72 „	„	Ditto, but no r. b. c.
	92 „	„	Ditto, but very few pale spir. found.
	119 „	Very slight motion?	Well stained.
	139 to 331 hrs.	Not examined fresh	None found (series of 8 bugs examined).

*Experiment V (a), at 24° C.*

Bugs fed on	Time when bug was examined after feeding	Appearances of spirochaetes in gut-contents	
		Fresh	Stained
2. iv. 1907	3½ hrs.	Most spir. fairly active, some sluggish	Mostly spirals, stain well, a few beaded and dividing; r. b. c. vanishing.
	6 "	Very slight intermittent motion, some motionless	Many rigid spirals, a few pale-staining, others with one half thick and fragmented chromatin; r. b. c. vanishing.
	8 "	Motionless, some short, others with globular swelling.	Ditto.
	22 "	Motionless	Many, some normal, others slender or pale and fragmented, no r. b. c.
	31 "	None found	Ditto.
	46 "	" "	Only 2 found, faintly stained, no r. b. c.
	72 "	Not examined	One bunch of spir. seen, fairly stained.
	96 "	" "	None found.

*Experiment V (b), at 24° C.*

27. v. 1907	6, 8, 24, 48, 96 hrs.	Practically same result as Exp. V: motionless after 8 hrs., no spir. after 48 hrs.	6 hrs., spiral and wormlike forms, these and r. b. c. stain well; 8—24 hrs. ditto, r. b. c. vanishing; 48—96 hrs., none found, no r. b. c.
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*Summary of the foregoing experiments.*

It will be seen from the foregoing protocols of the experiments that the spirochaetes still showed slight *movement* after

139 hours at 12° C. and were fairly active after 96 hours.
122 " 14° C.
42 " 16° C. and were fairly active after 28 hours.
6 " 20° C.
6 " 24° C. and were fairly active after 3½ hours.

In *stained films* the spirochaetes mostly appeared normal up to

278 hours at 12° C.
218 " 14° C.
19 " 16° C. though some were thick and beaded or irregular.
6 " 20° C. " " " "
3½ " 24° C. " " " "

No traces of spirochaetes could be detected in stained films after

139 hours at 20° C.
96 hours at 24° C.

Some *dividing forms* were seen after

8½ hours at 20° C.
8½ " 24° C.

*The red blood corpuscles* could still be discerned after

278 hours at 12° C.	
7122    „    14° C.	
42    „    16° C.	
81    „    20° C.	
8—24    „    24° C.	

In no instance could the spirochaetes be found in the coelomic fluid of the bugs although they were searched for in about  $\frac{2}{3}$  of the bugs tabulated in Experiments I—V.

#### *Experiment VI.*

This consisted in the *inoculation of mice* with the gut-contents of bugs maintained at a temperature of 12° C. after they had fed to repletion on the blood of a mouse suffering from infection with *Sp. duttoni*. The bugs fed at the height of the infection. The gut-contents of five bugs were used for the inoculation of each mouse, mixed with a drop of salt solution immediately before subcutaneous injection.

	Time when gut-contents of bugs were injected after bugs had fed on spirochaete blood	Time after inoculation when mouse showed first spirochaetes in its blood (examined every day)	Time after inoculation when the mouse died
Mouse I	21 hours	24 hours	7 days
„ II	51 „	48 „	19 „
„ III	95 „	168 „	28 „
„ IV	100 „	168 „	Recovered
„ V	120 „	72 „	28 days

Under ordinary conditions mice inoculated with *Sp. duttoni* show parasites in their blood after 14—20 hrs. They usually die on the 4th to 7th day. The sojourn in the bug for 21 hrs. at 12° C. does not seem to exert any influence on the spirochaetes, but after 51 hours there is a distinct delay with regard to the time when they appear in the blood of the mice and also with regard to the time when the mice die. This experiment establishes the fact that the spirochaetes survive for 5 days in the bug. The delay in their appearance in the blood of the mice may be due to the spirochaetes being present in reduced numbers or possibly to their having become attenuated. Judging from the results of Experiments I—VI it is probable that the spirochaetes may be infective after a still longer sojourn in the bug at a temperature of 12° C.

## CONCLUSIONS (I).

The foregoing experiments show that *Spirochaeta duttoni* retains its virulence after a sojourn of 5 or more days in the intestine of *Acanthia lectularia* when the insect is maintained at a temperature of 12° C. Living (motile) spirochaetes were observed in the gut-contents of the bugs up to 6 days at 12° C. but only up to 6 hours at 20—24° C. The observations made at various temperatures appear to indicate that the spirochaetes are simply digested by the bug and that their more rapid disappearance from the insect's gut at higher temperatures is dependent upon the insect's digesting its food more rapidly when kept warm. The disintegration of the ingested red blood corpuscles in the insect's gut runs parallel to that of the spirochaetes, though the latter can be found in stained specimens long after the corpuscles have disappeared and the spirochaetes themselves have ceased to give any evidence of life.

Typical longitudinally dividing forms were encountered in stained preparation made from the contents of the bug's intestine 3½ to 8½ hrs. after the bug had fed on spirochaete blood, when the insects were maintained at 24° and 20° C. respectively.

The rapid death of the spirochaetes in bugs maintained at 20—24° C. may account for the negative results of the infection experiments carried out by Breinl, Kinghorn and Todd. Although the bug is not the true host of *Sp. duttoni* it is conceivable that it may serve as a mechanical carrier of the parasite. It has been proved that different species of Trypanosomes may be transferred by means of the bites of blood-sucking flies (*Glossina*, *Stomoxys*<sup>1</sup>) and that the plague bacillus may be transferred by means of *Acanthia lectularia*<sup>2</sup> when only a short interval (some minutes or usually less than 24 hours) elapses between the successive bites of the insect. Such experiments still require to be carried out with the bug and pathogenic spirochaetes.

Incidentally I may add that prior to accidentally losing the strain of *Sp. duttoni* it was maintained with undiminished virulence by passage through 100 mice during the course of one year.

I am indebted to Dr Levaditi of the Pasteur Institute for the strain of *Sp. duttoni* (R. Koch's) with which these experiments were carried

<sup>1</sup> See Nuttall (1907), for a summary of this subject.

<sup>2</sup> See Verjbitaki (1908) in the references which follow.



out. Finally I should like to state that my laboratory assistant, Mr B. G. Clark, has been of material assistance to me in carrying out the foregoing investigation.

*Note on Experiments with Spirochaeta obermeieri.*

Since the foregoing pages went to press I have, through the courtesy of Geheimrath C. Fraenkel of Halle, succeeded in obtaining a strain of the true *Sp. obermeieri*, which had been brought from Russia. Although my experiments with this spirochaete have not been concluded, the results I have obtained appear worth recording, more particularly because a positive infection experiment with *Acanthia lectularia* was obtained.

*Observations on the behaviour of Spirochaeta obermeieri  
in the body of Acanthia lectularia.*

*Experiment I, at 12° C.*

Bugs fed on	Time when bug was examined after feeding	Appearance of spirochaetes in gut contents	
		Fresh	Stained
27. iv. 1908	6 hrs	Very active	Many rigid spirals, r. b. c. all staining well.
	22 „	Fairly active	Ditto, also a few worm-like.
	27 „	Sluggish	Ditto.
	47 „	Almost motionless	Ditto.
	71 „	? Motionless	Ditto.
	96 „	Motionless	Ditto, but r. b. c. begin to vanish.
	120 „	None found	None found, r. b. c. gone.

*Note:* One spirochaete found in coelomic fluid taken from leg after 6 hrs., the examination in all other cases proved negative.

*Experiment II., at 25° C.*

27. iv. 1908	3 hrs	Some very active others slow	Many rigid spirals, a few worm-like, r. b. c. all staining well.
	20, 27, 45 hrs	All motionless	Ditto, but r. b. c. fading.
	72 hrs	No spir. found	No spir., no r. b. c. found.

*Note:* No spirochaetes found in coelomic fluid from leg at any time.

*Summary of foregoing experiment.*

It will be seen from the foregoing protocols that the spirochaetes still showed *movement* after

27—47 hours at 12° C. but were motionless after 96 hours.

8 „ 25° C. „ „ 20 „

Parasitology 1

10

In *stained films* the majority of the spirochaetes appeared normal up to

96 hours at 12° C., they disappeared after 120 hours.  
45    „    25° C.    „    „    72    „

Some *dividing forms* were detected after 22 hrs. at 12° C.

The *red blood corpuscles* could be discerned after

96 hours at 12° C.  
45    „    25° C.

*A positive infection experiment by means of A. lectularia.*

3. iv. 1908. Seventeen bugs were allowed to feed on an infected mouse (A) showing many *Sp. obermeieri* in its blood. The bugs were allowed to feed partially for about 5 minutes and were then transferred directly to a normal mouse (B) upon which they fed to repletion.

6. iv. 1908. Eighteen bugs fed similarly on an infected mouse (C) and immediately afterwards on mouse (B).

4—14. iv. 1908. The blood of the mouse (B) was examined for spirochaetes on 11 successive days with negative results.

15. iv. 1908. Spirochaetes were found in small numbers in the blood of mouse (B).

27. iv. 1908. Mouse (B) was found dead, no spirochaetes were found on microscopic examination but their presence was proved by the inoculation of the mouse's spleen pulp and blood into another mouse, which showed spirochaetes in its blood 9. v. and died 10. v. 1908.

*Conclusions (II).*

The foregoing experiments, whilst not sufficiently numerous to permit of any final conclusions, appear to indicate that *Spirochaeta obermeieri* may die out more rapidly than *Sp. duttoni* in the gut of the bug. It is possible that the bugs digested their food more rapidly owing to their being more hungry than the lot used for experiments with *Sp. duttoni*. The experiments are being repeated.

It has been demonstrated by one experiment that *Acanthia lectularia* fed on an infected mouse and immediately afterwards upon a healthy mouse is capable of transmitting the spirochaete.

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THE STRUCTURE AND BIOLOGY OF *HAEMAPHYSALIS*  
*PUNCTATA*, CANESTRINI AND FANZAGO. I.

By GEO. H. F. NUTTALL, F.R.S., W. F. COOPER, B.A.,  
 AND L. E. ROBINSON, A.R.C.Sc. LONDON.

Plates XII—XVI.

INTRODUCTION.

THE importance of various species of ticks in relation to the propagation of protozoal diseases, is so generally recognised that it has appeared to us eminently desirable to make a detailed study of one of the common species. Such a study has seemed to us specially necessary for the reason that our knowledge of these parasites is very imperfect, in spite of the fact that they possess an economic interest of the first order. Some of the diseases which ticks transmit, notably those due to the haematozoal parasites belonging to the genus *Piroplasma*, are among the most devastating affections of domesticated animals in many parts of the world, the useful animals which suffer from piroplasmosis being cattle, sheep, goats, horses, and dogs. The disease known as "Heart-water," occurring in South Africa and affecting sheep, goats and cattle, is likewise tick-transmitted. A disease of the domesticated fowl, analogous to relapsing fever in man, likewise of economic importance and occurring in different parts of the world, has also been demonstrated to be transmitted from animal to animal through the agency of ticks. The fowl disease is due to a *Spirochaeta* which is conveyed by ticks; the same holds for human "tick fever" and a spirochaete infection in cattle occurring in parts of Africa. Recent investigation appears to have clearly established the fact that a tick conveys spotted or Rocky Mountain Fever to man. Moreover it has been claimed that a Nematode worm, the *Filaria perstans*, parasitic in man, undergoes its development in a tick which is capable of conveying the parasite from one human

host to another. There can be no doubt but that ticks will be found, upon further investigation, to be associated in the transmission of an increasing number of diseases in animals.

Ticks belong to the class *Arachnoidea*, in which are included the scorpions, spiders and mites, and almost all the members of the class possess eight legs when they reach the adult stage. Ticks constitute a well-defined super-family, the *Ixodoidea*, in the order *Acarina*, and in the light of recent knowledge may be divided into two families, the *Argasidae* and the *Ixodidae*.

It would be out of place to dwell here upon the particular characters possessed by the different genera, especially as these will be duly considered in a systematic work which is being prepared for publication.

The *Ixodoidea*, as far as is known, derive their nourishment entirely by sucking the blood of their hosts. These hosts are almost exclusively terrestrial vertebrates and include practically all classes of *Mammalia*, *Aves*, *Reptilia* (*Chelonia*, *Lacertilia* and *Ophidia*), and *Amphibia* (*Bufo*). Two species are recorded as attacking *Insecta* (*Coleoptera*)<sup>1</sup>.

The *Ixodoidea* are distinguished from other *Acarina* by their relatively large size; by the position of the spiracles (absent in the larva), situated as they are, in the posterior half of the body, behind the last pair of legs, or as in the *Argasidae* usually between the third and fourth pair of legs; and by the unique structure of the piercing and sucking mouth parts, complicated by the fusion of the basal segments of some of the perioral appendages so as to form a definite structure, the *capitulum*, articulating with the anterior end of the body. The *body* is compressed dorso-ventrally. The *capitulum* bears two pairs of jointed appendages. On the whole, the *Ixodoidea* are found to agree in their general morphology with the remainder of the order *Acarina*, in so much as the cephalothorax is indistinguishably fused with the abdomen, in the possession of an unsegmented body and in the character of the mouth parts.

The *Ixodidae* resemble each other in all essential characters, but differ from the *Argasidae* in the fact that a hard dorsal shield or *scutum*, absent in the latter, is invariably present in all stages; also the *capitulum* is visible from the dorsal surface in the former, whereas in the latter it is entirely concealed (excepting in the larval stage) by the overhanging anterior extremity of the body: the two families differ also in many respects regarding their habits.

The life-history of the *Ixodidae* may be briefly summarised as follows:—

<sup>1</sup> Neumann 1901, pp. 274 and 294.

The female lays her numerous eggs in the ground. The eggs hatch out in due course and the six-legged larvae issue. The larvae attach themselves to a host and engorge blood, after which, in some cases, they fall to the ground, in others, they remain attached to the host. In all cases, they moult after a time and the eight-legged nymph appears. The nymph either attacks a fresh host or reattaches itself to the original host; it feeds and when gorged drops off, or in certain species, retains its hold. A second metamorphosis takes place and the adult tick, male or female, issues from the nymphal skin.

Copulation takes place upon the host, in some cases (*Ixodes*) it may also take place upon the ground. The males suck blood in moderate amount (a fact often denied), whilst the females become greatly distended with blood which they digest, partially while on the host and partially after dropping to the ground, after which, they lay their eggs in due course, shrivel and die. We shall consider the life-history in detail in our study of *Haemaphysalis punctata*.

Although the genus *Ixodes* is usually regarded as typical of the *Ixodidae* to which it gives its name, we have, for reasons of convenience, chosen a species of the genus *Haemaphysalis* to work upon; we have been able to raise it without difficulty through its various stages under laboratory conditions; also, the species is sufficiently common in Europe to be readily obtained. Most of our material was collected in Kent, the gorged males and females being taken, for the most part, from sheep in the neighbourhood of Romney Marsh.

Before proceeding to a further consideration of the structure of *H. punctata*, we would state that, in common with other *Ixodidae*, the main distinctions between the different stages are as follows:—

The *larva* is a minute six-legged creature, possessing no respiratory (tracheal) system; it has no trace of sexual organs. The scutum covers but a part of the dorsum. When fully gorged, it is about as large as the nymph which subsequently issues.

The *nymph* is larger than the unfed larva; it possesses a tracheal system and a pair of spiracles; also an *Anlage* of the genital system, in the shape of a minute pit at the site of the future genital orifice. The scutum approximates to that of the female. When gorged, it is almost as large as the adult which issues from the nymphal skin. We have been unable to determine any sexual differences in the larval or nymphal stages, but it may be mentioned that nymphs have frequently been mistaken by various authors for "young" females.

The *adult* is larger than the nymph and possesses a well-developed

respiratory system and sexual organs. Both sexes of the ungorged adults are of about the same size; but when fully fed, the female greatly exceeds the male in size. The scutum in the male covers almost the entire dorsum; the anterior portion only, in the female.

In species of *Ixodidae* which possess eyes, they are present, as far as we know, in all the stages, but eyes do not occur at all in the genera *Haemaphysalis*, *Ixodes* and *Aponomma*.

We shall proceed to give a somewhat detailed specific description of *Haemaphysalis punctata*, together with the iconography and distribution.

### ***Haemaphysalis punctata*, Canestrini and Fanzago, 1877.**

#### SYNONYMY.

*Haemaphysalis punctata*, Canestrini and Fanzago, 1877.

*Haemaphysalis sulcata*, Canestrini and Fanzago, 1877.

*Rhipicephalus expositicus*, L. Koch, 1877.

*Herpetobia sulcata*, Canestrini, 1890.

The foregoing synonymy is given by Neumann (1897, p. 327). He includes *Haemaphysalis peregrinus*, Pickard-Cambridge (1889, p. 406), but on referring to the descriptions and figures of the latter author, we were unable to agree with Neumann, for the reason that it was impossible to recognise the tick from the inadequate description. The types, moreover, have been lost.

*Haemaphysalis cinnaberina*, C. L. Koch, 1844 and 1847, was degraded to *H. punctata* var. *cinnaberina* by Neumann (1905, p. 237), *vide infra*.

Canestrini (1890, p. 526) included *Ixodes chelifer*, Mégnin, 1880, and *Pseudixodes holsatus*, Haller, 1882, in this synonymy, but he was wrong in so doing, as was pointed out by Neumann (1897, pp. 338, 360).

Railliet (1895, p. 714) quotes Canestrini. Neumann (1901, p. 260) considers that *Ixodes testudinarius*, Murray (1877, p. 192), which Murray makes synonymous with *Ixodes marginatus*, Leach, to be probably *H. punctata*. He appears to base this opinion on a figure of Murray's which seems to us to be quite unrecognisable.

#### DESCRIPTION AND ICONOGRAPHY.

Canestrini and Fanzago's original description (1877, p. 121 repr. and 1877—78, p. 189) refers only to the ♂ and ♀, and is so brief that it would be impossible to identify the species by it. The description

given by L. Koch (1877, pp. 196—198) of *Rhipicephalus expositicus* certainly agrees very closely with that of *H. punctata*, but he gives no figure. Canestrini (1890, pp. 523—526) subsequently gave a more detailed and fairly accurate description, more especially of the ♀; he gives the first figures published (Pl. 41, Figs 6 and 6a), showing a ventral view of the ♂ and a ventral view of the ♂ capitulum. Although inaccurate, Canestrini's figures show the principal ♂ characters. All the points in his description, with the exception of a few measurements, are included in Neumann's description (1897, pp. 327—330). Berlese (1891, Fasc. 55, Pl. 1) gives a coloured figure of *H. sulcata*, together with outlines of the dorsal and ventral aspects of the capitulum and of the spiracle. To us there appears to be no doubt that he was dealing with a gorged nymph of *H. punctata*, although he omits the cornua on the basis capituli and places the chelicerae ventrally instead of dorsally in his figure of the capitulum. In a later publication (1891, Fasc. 58, Pl. 10) he gives an inaccurate coloured figure of *H. punctata* ♂, and equally inaccurate contour figures of the ♂ (ventral aspect), ♂ capitulum and palp, ♀ (dorsal and ventral), ♂ and ♀ spiracles. Finally, Neumann (1897, Figs. 1 and 2, p. 328) figures the hypostome accurately and gives dorsal views of the ♂ and ♀ chelicerae of the left side. His figure of the ♂ chelicera is correct (compare with our Pl. XIV, Fig. 4 and XV, Figs. 5, 6) but that of the ♀ is inaccurate, more particularly with regard to the so-called third apophysis (dorsal process). Wheler (1906, Figs. 30—32) has published photographs of the ♂ and ♀.

#### SPECIFIC DESCRIPTION.

##### Female (Plate XIII).

*Unfed.* Length (anterior margin of scutum to posterior margin) 2·73—3·23 mm. Breadth (maximum), 1·78—2·08 mm.

**Body**<sup>1</sup> flattened, contour ellipsoidal; reddish-brown dorsally, paler ventrally; glabrous (few excessively minute scattered hairs); *marginal grooves* deep, sharply defined, extending from external angles of scutum, anteriorly, to 4th festoon on either side, posteriorly: *postero-median groove* shallow, extends from median festoon almost to posterior margin of scutum; *accessory grooves* as long as *postero-median groove*, wide and shallow: *foveae* opposite legs IV, darker in colour than surrounding

<sup>1</sup> Refer to text figures 1, 2, p. 165.



parts: *festoons* rectangular, twice as broad as long, nos. 1, 2, and 3 only, on each side, divided by the marginal groove from rest of body. *Scutum*, length 1.08—1.37 mm., breadth 1.05—1.31 mm.; broadly elliptical with flattened postero-lateral margins; dark reddish-brown with darker irregular markings; deeply emarginate anteriorly for reception of basis capituli; surface polished, traversed by minute irregular radiating furrows; punctations irregularly scattered, few in number: *cervical grooves* deep anteriorly, shallow and wider posteriorly, almost parallel throughout their length. *Venter*:—*spiracles* nearly circular, external margin flattened, macula large and slightly excentric: *genital grooves* divergent, do not quite reach festoons posteriorly: *genital orifice* opposite interspace between Coxae II and III: *anus* nearly circular; *valves*<sup>1</sup> punctate and bearing 5 minute hairs on each: *anal groove* well-marked, bifurcated at its anterior extremity in the form of a Y, the forks of which encircle the anus on either side and terminate in the genital grooves.

*Capitulum* (see text figs. 3—9, p. 169 et seq.): length, 770—880 $\mu$  (from line joining tips of palps to line joining extreme postero-lateral angles of basis capituli): *basis capituli* rectangular, cornua obsolete; *porose areas* large, well separated, rather wider than long; ridge on ventral surface semicircular in contour: *palps* valvate, sub-conical, truncated; article 1 small, almost entirely concealed within the basis capituli, article 2 contracted proximally, much widened out distally with a prominent ridge running round its ventral, lateral and dorsal surfaces and forming the external angle of the palp; infra-internal margin furnished with a continuous fringe of feather-like hairs, usually thirteen in number but subject to some variation; three similar hairs on corresponding margin of third article, the ventral surface of which is produced as a large pointed backwardly directed protuberance; article 4 small and stumpy, with a tuft of stiff hairs on its free extremity, Neumann (1897, p. 328) gives the number of these hairs on the fourth segment of the palp as six; we have found eight to ten such hairs, but the number does not appear to be by any means constant): *chelicerae*: *internal article* with large base and prolonged as a single cutting-lancet with an outwardly turned point; *dorsal process* crescentic with the points folded towards each other; *external article* a flattened plate with four (sometimes five) outwardly directed cusps, the first of which is the smallest, the others gradually increasing in size to the largest

<sup>1</sup> Neumann (1897, p. 328) says that the anal valves are punctate, but without hairs: we have invariably found five minute hairs on each valve in addition to punctations.

proximal cusp: *hypostome* spatulate, five files of sharply pointed teeth on each half (we have found a slight degree of variation in this respect, the specimen selected for the figure, Pl. XIV, Fig. 2, possessing an irregular sixth file on the right half of the hypostome; we have also seen a female of *H. punctata* possessing six complete rows of teeth on either half of the hypostome); the anterior, median and posterior teeth are smaller than the rest; *corona* well-developed, studded with minute denticles (the corona is frequently curled over on to the dorsal surface of the hypostome and may escape observation if the curling is extreme).

Legs of moderate length, dark-brown, strong; *coxae* slightly longer than wide, each bearing a short wide spur at the posterior margin, near the internal angle; *tarsi* relatively short, spurred, the single spur on each being terminal: *pulvilli* of medium size; two-thirds length of claws. (Canestrini (1890) gives the length of the legs as follows: Pair I = 2.95 mm., II = 2.50 mm., III = 2.5 mm., IV = 2 mm.)

*Gorged* (Pl. XIII). *Body* oval, more or less distended, may attain a size of 12 × 7.5 mm. (Canestrini, 1890); colour variable—light steel-grey, slate-grey, brownish or brownish-black, yellowish streaks and irregular markings present according to the condition of distension of the Malpighian tubes; dorsal and ventral surfaces punctate: *marginal grooves* and *festoons* obliterated; *postero-median* and *accessory grooves* well defined; anterior to these, four short almost parallel grooves, the outermost of which are often interrupted at the middle of their length (Pl. XIII). *Venter*:—*post-anal groove* not so sharply defined as in unfed ♀ and its bifurcated anterior extremity is obliterated: two dimple-like depressions, one on either side of post-anal groove: *genital orifice* slightly anterior to level of *coxae II*, but position varies according to the state of engorgement, being pushed more and more forward until oviposition commences, when it lies nearly at the base of the capitulum. Otherwise as in unfed ♀.

#### Male (Plate XIII).

*Unfed*. Length (measured as in female) 2.6—3.27 mm., breadth (maximum) 1.67—2.04 mm.

*Body* ellipsoidal in contour, wider behind than in front, convex dorsally, concave ventrally: *scutum* covers entire dorsal surface; dark reddish-brown with darker, almost black irregular markings; glabrous and polished; punctations fine, numerous, regularly distributed; *scapular angles* narrow; *cervical grooves* deep anteriorly, shallow and divergent posteriorly; *marginal grooves* narrow, sharply defined,

terminating anteriorly opposite leg II, posteriorly at 4th festoon; *accessory grooves* shallow, deeper at posterior extremity; *foveae* in small depressions opposite interspace between legs III and IV. *Venter*:—*spiracles* oval, wider in front than behind, external margin almost straight, postero-marginal angle pronounced; *macula* small, situated towards antero-internal angle: *genital grooves* almost parallel anteriorly, divergent behind coxae IV, extend to posterior marginal festoons; *post-anal groove* straight, bifurcated at its anterior end as in female: *festoons* sharply divided from body by a deep groove: *genital orifice* opposite coxae II: *anus* as in female.

**Capitulum**, length 500—670 $\mu$  (measured as in female): *basis capituli* rectangular, relatively longer than that of female; dorsal ridge with salient rounded angles (cornua) at its extremities; few coarse scattered pores on site of porose areas of female: *palps* relatively shorter than those of the female, more conical and less truncated; retrograde spine on ventral surface of article 3 more pronounced: *chelicerae*—*internal article* as in female; *dorsal process* with single large outwardly directed cusp from which a thin strap-like process runs down the dorso-external margin of the internal article and becomes indistinguishably fused with it; *external article* small, with three cusps, the most proximal of which is large and strong: *hypostome* less spatulate than that of female, otherwise similar.

**Legs** I—III with well-marked blunt *coxal spurs*; leg IV with elongated sabre-shaped coxal spur directed backwards and curving inwards, equal in length at least to that of the coxa: a short blunt backwardly directed spur on the dorsal side of the trochanter of leg I; *tarsi* as in female; Neumann (1897) observes that the tarsi IV of the ♂ *Haemaphysalis punctata* are bicalcarate, a point which we have failed to confirm.

**Gorged** (Pl. XIII). Alteration so slight as not to necessitate description.

#### Nymph (Plate XIII).

**Unfed.** Length: tips of palps to posterior margin 1.39—1.55 mm. (measured as in adult), breadth 1.12—1.31 mm.

**Body**—oval, flattened, colour varying from pale yellow to brownish-red: *scutum* sometimes broader than long (length 0.37—0.55 mm., breadth, 0.47—0.57 mm.); more rounded than that of ♀; few scattered punctations; divided into minute polygonal areas by a fine reticulum of superficial fissures; otherwise like that of ♀: *grooves* on dorsum similar

to those of ♀: *foveae* extremely small. *Venter* resembles that of female, the *genital orifice* being represented by a small imperforate pit: *spiracles* almost circular; *macula* excentric, situated towards the inner margin of the spiracle.

**Capitulum**—length (from tips of palps to postero-dorsal ridge) 0·23—0·29 mm.: *basis capituli* widest at base of palps; dorsal surface extended laterally on either side as a triangular process with rounded point; *porose areas* absent: *palps* longer than hypostome, of elongated triangular form; prominent external angle on article 2, which bears on its infra-internal margin a fringe of about 5 feather-like hairs; article 3 carries a single feather-like hair in a corresponding position: *chelicerae*—*digit* as in female: *hypostome* slightly spatulate; two files of teeth on each half.

**Legs** paler in colour than in adult; no tarsal spurs; otherwise as in adult.

**Gorged.** The changes undergone by the nymph during engorgement are similar to those of the ♀, viz. obliteration of the grooves on the surface of the body, alterations in colour, etc. When replete the nymph measures 3·3 × 2 mm.

#### Larva (Plate XII).

**Unfed.** Length (tips of palps to posterior margin) 0·58—0·70 mm., breadth (maximum) 0·43—0·48 mm.

**Body** oval, wider posteriorly, less flattened than other stages; colour dull ochre-yellow; few pale-coloured hairs, regularly arranged (see Figs. 1 and 2); *scutum* broader than long (length, 0·25 mm., breadth, 0·32 mm.); reddish-brown in colour, contour follows that of body in anterior half, postero-lateral margins almost straight, with large rounded postero-median angle; surface reticulated as in nymph; *cervical grooves* deep, straight and not extending to posterior margin: *foveae* absent; *postero-median* and *accessory* grooves faintly marked. *Venter*:—*post-anal groove* slight; *genital grooves* absent: *spiracles* absent.

**Capitulum**, length (tips of palps to dorsal ridge) 0·15 mm.: *basis capituli* as in nymph but lateral angles less pronounced: *palps* triangular, external angle not prominent; all articles fused together with the exception of the 4th, which is relatively larger and more terminal in position than in the other stages; a single feather-like hair is carried on the infra-internal margin: digits of *chelicerae* as in nymph and female: *hypostome* with four files of teeth, two on each half.

**Legs**, pale ochre-yellow; no spurs on coxae or tarsi; *pulvilli* relatively larger than in other stages.

**Gorged.** Beyond the distension of the body, the changes during and after engorgement in the larva do not call for any special reference. A noteworthy feature is the fact that larvae of *H. punctata*, taken from inside the ears of rabbits, when fully gorged are a clear straw-yellow in colour, from which it would appear that lymph and not blood had been ingested.

***Haemaphysalis punctata* var. *cinnaberina* (C. L. Koch),  
Neumann 1905.**

This variety, based on a single young ♀, is distinguished from the typical *H. punctata* by the presence of a white chitinous circle round the genital orifice and also round the anus. The type, a dried specimen, from Pará, Brazil, is in the R. Lucas Collection (Berlin Mus.) where it was examined by Neumann (1897, p. 331), who first described it as *H. cinnaberina*, C. L. Koch, but subsequently degraded it to a variety. This tick was inadequately described and figured by C. L. Koch (1844, p. 237; 1847, p. 123 and Pl. XXVI, fig. 97).

**GEOGRAPHICAL DISTRIBUTION OF *HAEMAPHYSALIS PUNCTATA*.**

**HOSTS ATTACKED.**

Canestrini (1890, p. 525) and Berlese say that this species is fairly common on sheep in Italy; also on goats and fallow deer. L. Koch found a ♂ in a wood near Nürnberg, and named it *Rhipicephalus expositicius*. Neumann has males collected by Colin on a bull (Railliet Coll.): one ♂ and 4 ♀'s from Digne, Beaune, Corsica (Simon Coll.): 2 ♀'s and 1 ♂ from Villefranche found on a horse (Aveyron): 5 ♀'s and 6 ♂'s from sheep and ox at Ste. Jean de Luz: 1 ♀ and many larvae from sheep in England (B. A. I. Coll., Washington, D.C.): 1 ♀ on *Numenius arquata* and 1 nymph on *Otus otus* at Utrecht (Oudemans Coll.): 2 ♀'s at Jassy, Roumania (Leon Coll.): 1 at Fiume, Croatia (Simon Coll.): 1 found under stones and 1 ♀ on a lizard, *Acanthodactylus vulgaris* at Oran (Domergue): 1 ♀ on a bull at Blida, another on a goat at Medea, 1 from Marnia, 1 from a sheep in Egypt (Smithsonian Inst. Coll. Washington): 1 ♂ and 1 ♀ from Orotava, Teneriffe, and 1 ♀ from Funchal, Madeira (Kraepelin Coll. Hamburg Mus.): 5 ♀'s from Canary Islands (Paris Mus.): 1 ♀ from a horse in Aomori, Japan. According

to Neumann, *H. punctata* is sufficiently cosmopolitan but does not appear with frequency; the specimens collected are never very numerous. Canestrini and Berlese found nymph and larvae in Italy, on *Lacerta viridis* and in Dalmatia on *Lacerta muralis* var. *pelagosae*. Neumann's specimens came from a lizard in the Island of Cyprus, and from horse, hare, red partridge and grey partridge (Aveyron).

Since Neumann's description appeared, *H. punctata* has again been recorded in England by Pocock (1900, p. 326), who states that the specimens were collected by Mr F. Pickard-Cambridge at Dungeness, on a hedgehog and amongst shingle on the beach. Neumann (1901, p. 260) records 11 ♀'s, in 5 lots, of which one lot is at the Hamburg Museum and four at the Berlin Museum, which were derived from Athens, Crete, the Cyclades, Teneriffe and Japan: 9 nymphs found on *Lacerta ocellata* var. *tingitana*, were collected by Domergue at Djebel Ksel in Algeria: 3 nymphs were found on *Vipera aspis* by C. Parona at Genoa; 1 ♀ found on the hedgehog by Oudemans in Holland.

In the course of the last few years, we have received large numbers of specimens from Kent, especially the districts surrounding Lydd and Canterbury: the great majority of these were taken from sheep, but specimens taken from goats and ferrets have been received.

#### TECHNIQUE AND METHODS.

In the study of the external anatomy of *Haemaphysalis punctata*, the authors have examined living specimens; killed, cleared and mounted entire in various media; dissections and sections. The coloured figures are faithful reproductions of living specimens, the original drawings of which were made by means of the Abbe camera lucida and amplified by numerous sketches of mounted specimens.

The Zeiss binocular dissecting microscope has proved itself of the greatest value in the making of fine dissections and in the execution of sketches of the living tick.

Specimens intended for the study of the structure of the chitinous exoskeleton were usually treated with a cold 10 % solution of potash for some hours, or until the soft parts were entirely dissolved, washed in distilled water containing a trace of acetic acid, stained with picric acid, orange G or osmic acid (1 % aqueous solution), dehydrated by transference through graded alcohols, cleared in xylol or oil of cloves and mounted in Canada balsam. It was found that the chitin was efficiently stained by immersion for one minute in xylol saturated with

picric acid, immediately before the final clearing. Absolute phenol has been of great use in clearing entire specimens, the procedure being to melt a small quantity in a bottle or a watchglass in the embedding oven; drop the specimen in, leaving it until sufficiently cleared; transfer to xylol and mount in Canada balsam. The advantages of this method are the saving of time, the clean results, and the fact that the specimen is completely cleared before the soft parts are disintegrated—a useful feature in the study of the relationship between the exoskeleton and the internal structures.

Glycerine, glycerine jelly and especially monobromide of naphthalene have been used with advantage as mounting media in the study of the structure of the almost colourless, highly refractive chitinous parts, such as the digits of the chelicerae and the pulvilli. Minute dissections were made, as a rule, in oil of cloves, a method which facilitates manipulation with mounted needles. In the preparation of material for section cutting, all the better known fixing reagents have been tried, but the majority rendered the chitin so brittle that success was impossible. The reagent which has given satisfactory results is picro-sulphuric acid (Kleinenberg's formula). This is used in the undiluted condition and is heated to about 90° C. before immersing the material. The time required for complete penetration varies, but gorged females of *H. punctata* have been found to be thoroughly permeated with the reagent after an immersion of 1—2 minutes.

The authors intend to consider the subject in greater detail in the section dealing with the internal anatomy and histology.

For many purposes, photomicrography has lent assistance, but with few exceptions drawings alone have been used for illustration.

In the work on the structure of the external parts, considerable difficulty was experienced in obtaining proper orientation and fixation of the more minute parts for the purpose of drawing: this led the authors to try various plastic materials in which the part to be investigated could be lightly embedded and then orientated under the microscope, by means of mounted needles. The preparation known as Plasticine has proved an admirable medium for this purpose, and its use can be recommended in all cases where it is desirable to examine opaque objects which, on account of their form, are difficult or impossible to fix in proper position without resort to some such means.

## TERMINOLOGY.

One of the first features which strikes a reader, in perusing the published literature, is the hopeless confusion wrought by the loose use of terms, taken for the most part from the nomenclature used in the description of other classes of the animal kingdom, more particularly the Insecta.

As an example one might mention the *hypostome*, to which names innumerable have been applied, many of them being unsupported by morphological relationship; thus creating erroneous impressions of the homologies of this structure. Thus the *hypostome* has been called:—

- |                                 |                       |
|---------------------------------|-----------------------|
| (1) <i>Lingua.</i>              | (5) <i>Maxilla.</i>   |
| (2) <i>Maxillo-labial dart.</i> | (6) <i>Labium.</i>    |
| (3) <i>Glossoides.</i>          | (7) <i>Radula.</i>    |
| (4) <i>Ligula.</i>              | (8) <i>Languette.</i> |

With a view of obviating some of this confusion, we have been reluctantly compelled to add more terms to the already excessive number: in other cases a selection has been made from the existing nomenclature of those which have appeared to be the most suitable.

A list of the terms used, together with synonyms, will be appended at the end of this work.

## General Body-form.

*Haemaphysalis punctata* presents no marked structural differences from the rest of the *Ixodidae*. The body is roughly elliptical in contour, flattened dorso-ventrally, slightly concave on the ventral surface and convex on the dorsal surface in unfed specimens. It shows no differentiation into *cephalothorax* and *abdomen*. Deeply implanted into a special opening at the anterior end of the body is the *capitulum*: this structure, frequently referred to as the "head," is not such in a morphological sense, the true head of the tick comprising parts lying posterior to this and indistinguishably fused with the rest of the body. The *capitulum* is a very specialised structure, freely articulated with the body and bearing the oral opening together with its accompanying appendages. The *mouth* is not visible on external examination, being concealed between the median *hypostome*, ventrally, the *palps* laterally, and the paired *chelicerae*, dorsally. The *palps* are situated on either side of the *hypostome* and *chelicerae* and when adducted, ensheath and protect these medianally-placed appendages.



Key Figures to nomenclature of parts.

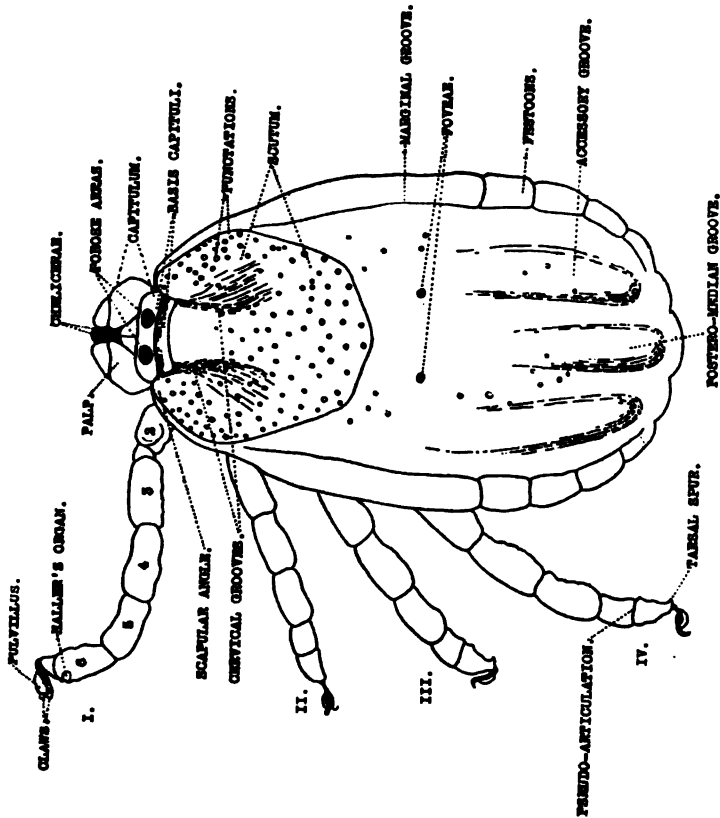


Fig. 1. *Haemaphysalis punctata*. ♀. Dorsal aspect showing the principal external features. The capitulum, on account of its downward inclination, is foreshortened. L. E. R.

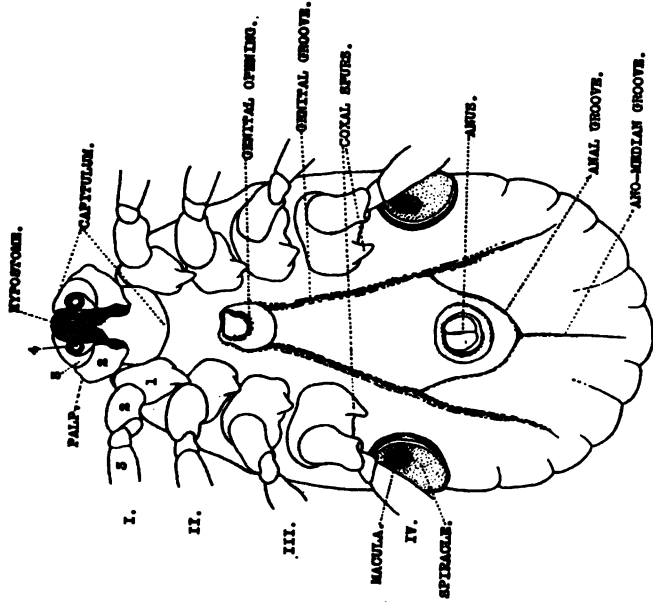


Fig. 2. *Haemaphysalis punctata*. ♀. Ventral aspect. L. E. R.  
The roman numerals indicate the legs, the arabic figures the segments, of the appendages.

It may be said that the body shows no marked trace of segmentation in either the nymphal or adult stages, but in the larva at any rate, the arrangement of the hairs and pores indicates a certain amount of metamerism; possibly the crowding of these tegumental structures in the later stages of the life-history may obscure any such appearance. The regular postero-marginal indentations of the body-contour in all the stages also seem to point to a feeble persistence of segmentation of the abdomen. We have reason to believe, after an examination of the embryo of this species, that at an early period of the development, some five or six segments appear in the body, posterior to the last pair of limb-buds. The exact significance of these phenomena we hope to determine later, when we have an opportunity of devoting more time to the embryology of the *Ixodoidea*<sup>1</sup>.

The dorsal surface of the body is covered almost entirely in the male and the anterior half only in the female, nymph and larva, with a plate of chitin which is denser, harder and more deeply pigmented than the general body chitin; this structure which is present in all ticks with the exception of the *Argasidae* is termed the *scutum*.

In many genera of ticks, the scutum bears the single pair of eyes, one on either lateral margin; in *Haemaphysalis*, however, as in the genera *Ixodes* and *Aponomma*, no trace of these organs exists.

On the ventral surface of the body in its anterior half are borne the legs, four pairs in the adult and nymph, and three pairs in the larva. Each leg is comprised of about six articles, all of which are freely movable with the exception of the most proximal article or *coxa*. Each of the legs carries at its extremity a foot provided with two long curved claws (*ungues*) and a pad (*pulvillus*).

On the ventral surface of the body in the adult, two large openings are seen in the middle line. The first lies midway between the coxae of the first and second pairs of legs and has the appearance of a transverse slit: this is the *genital orifice*. In the nymph, a minute pit is visible in a corresponding situation which may be considered as an *Anlage* of the genital orifice of the adult. The larva exhibits no trace of such a structure. Some distance posterior to the genital opening, at about the commencement of the posterior third of the body, is the *anus*, a longitudinal slit bounded laterally by a pair of crescentic plates, the *anal valves*, the whole being surrounded by a ring or annulus of thickened chitin.

<sup>1</sup> Wagner J. 1892, p. 319 "*Hinter den Beinen liegen 5-6 Mesodermgruppen.*"

The surface of the body exhibits a number of constantly occurring and well-defined grooves: these are the external indications of the lines of insertion of bands of muscle-fibres which run in a dorso-ventral direction through the body and are attached at either end to the chitinous cuticle. On the ventro-lateral margins of the body, posterior to the coxae of the fourth pair of legs, are situated the paired *spiracles*, a pair of more or less circular, slightly raised plaques of paler colour than the surrounding integument: in the larva, the spiracles and tracheal system are not developed. The posterior contour of the body is deeply indented at regular intervals by short grooves; these run round the margin from the dorsal to the ventral surface and define certain small rectangular marginal areas which are termed *festoons* in descriptive nomenclature. It is worthy of notice that the festoons are frequently more or less obliterated in the female and immature stages, by the distension of the body after engorgement, but although the alteration is not so marked in the male, the appearance of the festoons differs considerably in the unfed and gorged individuals (Pl. XIII). The *cuticle* of the tick is perforated by numerous pores of various forms which serve as a means of communication between certain modified hypodermal cells and the exterior. Numerous hairs are distributed over the different parts of the body; these differ among themselves according to their various functions. Certain areas of the body are glabrous.

The sensory organs are comprised of (1) certain defined areas into which numerous fine pores open, and beneath which the hypodermal layer is specially modified: such organs are found in the female on the dorsal surface of the basis capituli (*porose areas*), and in both sexes on the dorsum, where a pair of minute perforated areas are found, to which the name of *foveae* has been applied; these foveae the authors have found to exist in the nymph but not in the larva: (2) *Haller's organ*, a complex cavity in the cuticle of the tarsal portion of the first pair of legs, which communicates with the exterior by a minute slit-like pore and bears on its floor a number of sensory hairs, but does not contain otoliths<sup>1</sup>: (3) *tactile hairs* of different forms, arranged either in a scattered manner or collected into groups.

The tactile sense appears to be the most highly developed, after that the olfactory sense, and in spite of the absence of eyes, as found in some genera of *Ixodoidea*, *H. punctata* is very sensitive to light and in-

<sup>1</sup> We intend to publish an account of the general structure of "Haller's Organ" in different species, in the near future.

variably attempts to remove itself as far as possible from a source of light if exposed.

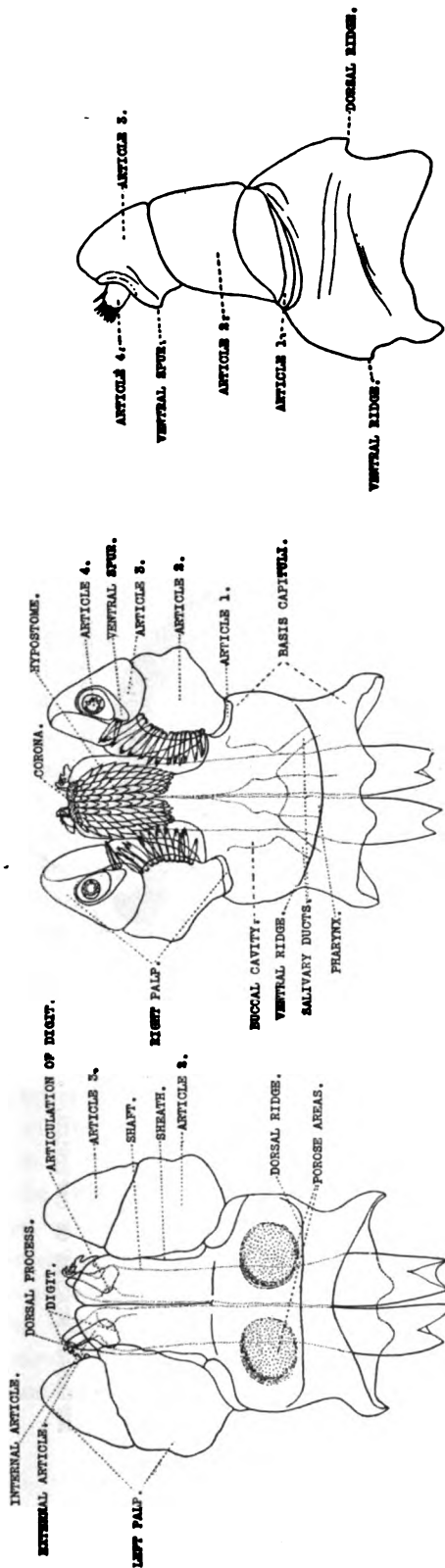
It must be borne in mind, especially in the reading of specific descriptions of *Ixodoidea*, that the general appearance undergoes very considerable transformations during and after engorgement; this is most noticeable in the larva, nymph and female, but the male may be considerably altered (see Pl. XIII). In the matter of colour, the gorged female exhibits any colour from pale greyish-yellow to almost pitchy-black. Prolonged immersion in preservatives, especially in alcohol, may completely modify the original colour, gorged females of *H. punctata* usually acquiring a more or less uniform dark reddish-brown tint.

### I. The Capitulum of the Female.

(Plate XIV, Figs. 1 and 2, Text Figs. 3—5.)

The capitulum is the head-like structure, situated at the anterior extremity of the body, upon which is borne the oral opening with its accompanying appendages. The large basal portion, hereafter spoken of as the *basis capituli* (b.c.), is constricted in its posterior half and this neck-like portion is telescoped into the anterior opening of the body cavity. The anterior portion of the basis capituli is roughly rectangular in shape, with flattened surfaces dorsally and laterally, and somewhat convex ventrally. A pronounced salient ridge, the *dorsal ridge* (d.r.), runs transversely across the dorsal surface, which, when the capitulum is raised in a line with the body-axis, comes in contact with the anterior margin of the scutum, the posterior constricted portion being concealed beneath the latter. Ventrally, the anterior portion of the basis capituli is differentiated by another ridge, the *ventral ridge* (v.r.), running in a transverse direction and curved, with its convexity directed posteriorly but not so sharply raised as the dorsal ridge. The sides of the basis capituli are flattened and the dorsal and ventral ridges are not continuous.

At the antero-lateral angles of the basis capituli, deeply implanted into a pair of large rectangular fenestrae, are the *palps* (p.). The ventral surface of the basis capituli is produced anteriorly in the median line as a spoon-shaped prolongation beneath the mouth; this structure is termed the *hypostome* (h.). On its dorsal side, the basis capituli is prolonged between the palps as a pair of parallel tubes which ensheath the *chelicerae*. The dorsal surface shows a pair of large shallow saucer-like



Figs. 3 and 4. *Haemaphysalis punctata*. ♀. The capitulum seen from the dorsal and ventral aspects respectively. L. E. R.

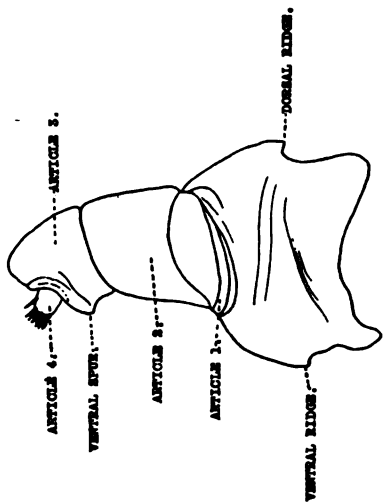


Fig. 5. *Haemaphysalis punctata*. ♀. Lateral aspect of the capitulum. L. E. R.

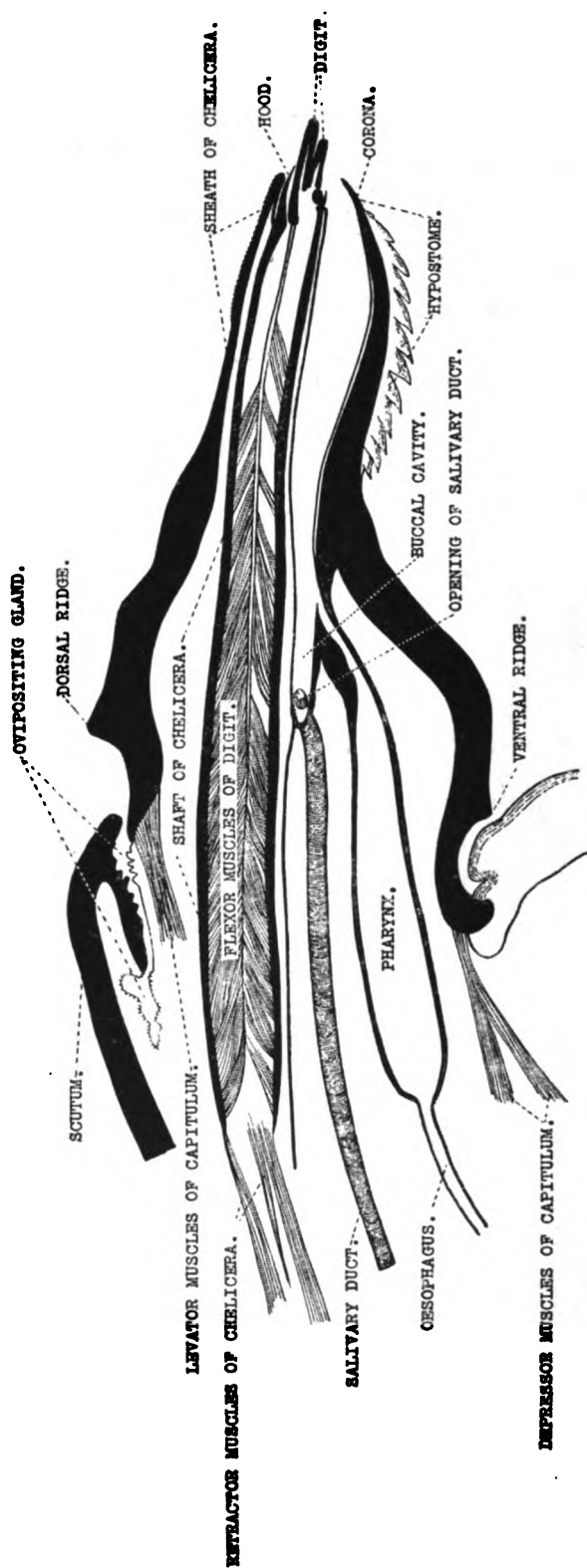


Fig. 6. *Haemaphysalis punctata*. ♀. Median longitudinal section through the capitulum showing the relations between the internal and external parts. Slightly schematized. G. H. F. N. and L. E. R.

depressions into which numerous minute pores open, hence termed the *porose areas* (p.a.); these structures will receive more detailed consideration later, under the heading of organs of the senses. With the exception of two small patches on the ventral surface, beset with minute stumpy hairs, the basis capituli is glabrous.

The chitinous integument of the basis capituli is thick and strong, and is thickened in internal ridges which give the whole structure stiffness and serve for the attachment of the muscles which bring about the movements of the capitular appendages and the *pharynx*.

The *hypostome* (h.), already referred to, is spatulate in outline and consists of a small proximal portion, whose ventral surface is smooth and convex, and a larger distal portion provided on its ventral surface with ten longitudinal files of pointed denticles which overlap one another from before backwards, in the manner of roof-tiles. It is divided along the median line by a fissure, separating the denticles into two series of five files, each file consisting of about twelve denticles. Although the number and arrangement of these denticles are fairly constant, a certain amount of irregularity frequently prevails, especially at the distal and proximal extremities. The denticles differ in size, the anterior and posterior being the smallest and the lateral being the largest. At the free extremity of the hypostome, is a thin plate of chitin continuous with the body of the hypostome, variable in size and studded with minute irregularly arranged denticles; this structure, the *corona* (c.), appears to be relatively larger in the immature stages. The upper surface of the hypostome is concave in a longitudinal direction and convex transversely; running down the median line is a small gutter (see transverse sections Plate XVI, Figs. 1—5), which, when opposed to the sheaths of the chelicerae above, forms a tube leading back to the buccal cavity. A longitudinal ridge runs along the lateral part of the ventral surface of each chelicerel sheath and a pair of slight ridges on the dorsal surface of the hypostome fit within the former, thus effecting a complete lateral closure of the space between the hypostome and chelicerae during the action of sucking (Plate XVI, Figs. 2, 3).

The *chelicerae* (Plate XV, Fig. 4 and Text Figs. 7—9) consist of a pair of more or less cylindrical appendages situated above the mouth. Each consists of a stout external *sheath* (sh.) already described as an anterior prolongation of the basis capituli, within which the chelicera proper lies, and is capable of complete retraction and of a considerable amount of protrusion: the two external sheaths lie in contact with one another throughout their length. The chelicera

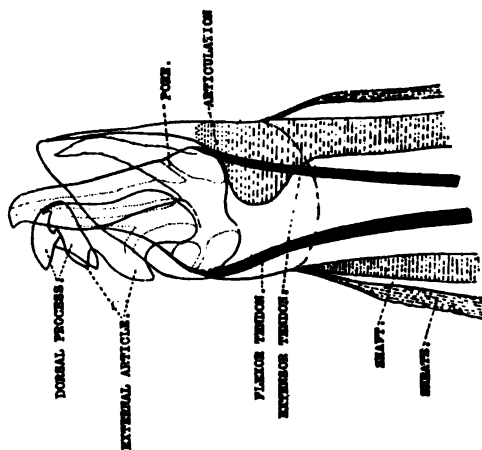


Fig. 7. *Haemaphysalis punctata*. ♂. The distal extremity of the right chelicera, showing the articulation of the digit, etc. L. E. R.

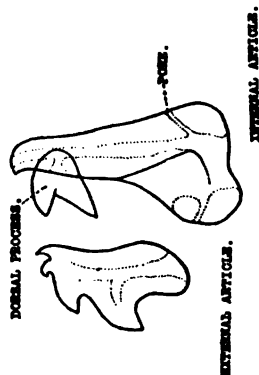


Fig. 8. *Haemaphysalis punctata*. ♂. Right chelicera, the articles isolated. L. E. R.

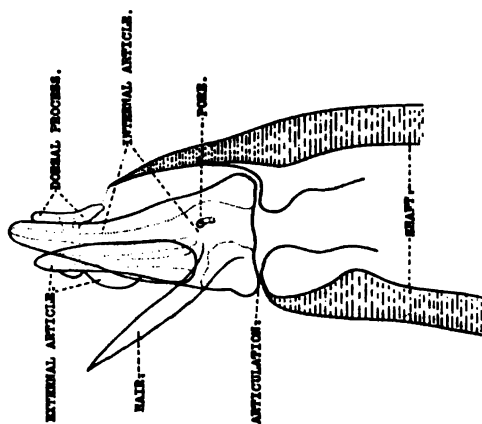


Fig. 9. *Haemaphysalis punctata*. ♂. Right chelicera, median or internal aspect. L. E. R.



proper consists of a long cylindrical tube, the *shaft* (s.ch.), upon the distal and free extremity of which is borne the chelate *digit* (d.ch.) which constitutes the cutting apparatus by means of which the animal penetrates the skin of its host. The sheath is covered on its dorsal and lateral surfaces, excepting its proximal third, with numerous minute reflexed denticles, regularly arranged in oblique rows and in close contact with one another, giving it an appearance which has suggested the term "shagreened sheath": it is firmly fused with the basis capituli at its proximal end. At its free extremity, the sheath is invaginated and becomes membranous and flexible, and this thin membranous portion is attached to the shaft some little distance posterior to the articulation of the terminal digit, by which arrangement the shaft draws in the extremity of the sheath over the delicate digital structures when fully retracted. The shaft of the chelicera is very long, so long in fact as to project backwards beyond the basis capituli into the body-cavity: it is divided into two portions, differing in diameter. The proximal portion, comprising about one-third of the entire length, has thinner walls than the distal portion and is dilated: the edges of the proximal extremity are irregularly incised and it is to this extremity that the retractor muscles of the chelicera are attached (Text Fig. 6): this dilated portion is occupied by muscles attached to its internal surface and from which proceed the tendons which are inserted into the base of the digit. The distal two-thirds of the shaft is roughly cylindrical with a slight ridge along its supra-internal margin, the ridge being longitudinally traversed by a minute canal (Plate XVI, Figs. 2—9): the chitinous walls are thick and show a delicate transverse striation: the distal extremity is thickened internally and presents a surface for the articulation of the base of the digit. At the junction of the proximal and distal portions of the shaft is a somewhat rectangular area of thinned and membranous chitin, in length about equal to the diameter of the shaft and extending round the dorso-lateral surface of the latter for about half its circumference. The *digit* consists of two movable articles, a larger *internal article* (i.a.), with which is articulated a smaller *external article* (e.a.). The internal article is expanded at its base and articulates directly with the shaft; it is prolonged forwards on the internal side in the form of a long cutting lancet with a slightly hooked extremity, the point of which is directed laterally; it is hollow throughout its length, the cavity in the expanded base being of considerable size. Near the distal extremity of this article, attached to its dorsal surface, is a crescentic chitinous structure with laterally directed points which is

often referred to as the *third apophysis*: it is firmly fused to the internal article, a prolongation of the cavity of which extends into it, and we consider it to be simply an outgrowth of the latter, comparable to similar processes often seen on the homologous articles of the chelicerae in the *Gamasidae* and *Oribatidae*, and for this reason we prefer to term it the *dorsal process* (d.p.). The external article articulates with the expanded base of the internal article; it consists of a flattened plate of chitin, the external margin of which is deeply incised, so as to form a series of four or five pointed cusps, of which the terminal one is the smallest, and the proximal the largest: it is hollow and its internal cavity is continuous with that of the internal article; indistinct channels appear to run out from the central cavity for a short distance along the lateral cusps. In many cases, the cavity of the internal article appears to terminate in a minute pore at the distal extremity; whether this is actually the case we are not in a position to express a definite opinion: it is, however, certain that the interior of the expanded base of the internal article communicates with the exterior by at least two fine channels, one on the internal side and one on the external (pr.), the possible function of which it is difficult to imagine.

The digit of the chelicera is surrounded on its internal side by a delicate hood of thin and very transparent chitin, a prolongation of the shaft of which it is a part; it is tapered away on the external side of the digit in such a manner as to fully expose the cutting parts of the appendage. The internal article bears on its basal portion a single serrate hair, which protrudes between the hood and the lancet-shaped portion of the former (see Text Figs. 7 and 9 and Pl. XV, Figs. 4—6).

The base of the digit, formed entirely from the expanded portion of the internal article, articulates with the shaft by a rocker-like hinge-joint, the shape of the articular surfaces being such as to limit the movement of the digit to a horizontal plane. The movements of the digit are produced by the tendons, one internal and one external, which originate in the mass of muscle occupying the cavity of the shaft (see Text Fig. 6, p. 170): the internal tendon is inserted into the internal article at its proximo-internal angle, while the external tendon, the stouter of the two, runs partially round the lateral surface of the article and is inserted towards the upper surface of the expanded base.

*The palps.* These appendages are inserted into the basis capituli at its antero-lateral angles. The general outline of each is that of a truncated cone; the basal portion is much constricted laterally, but not

in the dorso-ventral direction. Each palp is quadriarticulate, but the articles differ greatly in size and form. The proximal or *first article* is very small and is almost entirely hidden within the basis capituli; it is narrow from side to side, elongated in the dorso-ventral direction, and obliquely inclined to a plane at right angles to the long axis of the capitulum in such a manner as to bring its ventral portion posterior to the dorsal portion: externally it is only visible ventrally, being completely hidden on the dorsal side by a scroll-like fold of chitin derived from the basis capituli. The second and third articles constitute the greater portion of the palp. The *second article* is irregular in shape, very convex on its ventral, external and dorsal surfaces and is provided with an oblique ridge (r.) which runs forwards and outwards from the proximo-internal angles and which defines another surface directed backwards. The *third article*, smaller than the second, tapers distally to a blunt rounded extremity which forms the extremity of the palp; on its ventral surface, a little toward the internal margin, it bears a large pear-shaped fossa bounded by a raised margin, the latter being produced postero-internally into a large blunt salient process; in this fossa the comparatively minute *fourth article* is implanted. This article is very short and stumpy and is furnished with a crown of eight to ten short stiff hairs on its distal extremity. The interno-lateral aspect of the second and third articles of the palps is deeply concave, so that when adducted, the two palps ensheath the chelicerae and hypostome. The infra-internal margin of the palp is furnished with a single row of peculiar flattened feather-like hairs (Pl. XV, Fig. 1); of these hairs, thirteen to fifteen are carried on the second article and about three on the third article: they are directed towards the mid-ventral line of the capitulum, the tips of those of each side almost meeting when the palps are adducted. The surface of the palps is furnished with a few simple hairs and pores.

The *buccal cavity*. The buccal cavity is contained within the anterior part of the basis capituli; it lies between the bases of the palps, behind the hypostome, and is visible through the walls of the basis capituli in cleared specimens as a chitinous mass with a symmetrical outline of somewhat complex shape (the contour is indicated in Pl. XIV, Figs. 1, 2)<sup>1</sup>. The walls of the basis capituli, bounding the anterior

<sup>1</sup> On account of the prevailing system of making drawings from specimens cleared and mounted in Canada Balsam or other highly refractive medium, without reference to the untreated specimen, the outline of the buccal cavity and other internal structures have frequently been figured by different authors in illustrations to specific descriptions of the *Ixodidae*. As such figures are usually supposed to represent external structures, the inclu-

margin of the buccal cavity immediately behind the hypostome, are of great thickness, giving increased strength to the anterior part of the basis capituli and firm support to the bases of the mouth appendages; running through these thick walls, ventral to the buccal cavity, is a pair of narrow canals leading to the bases of a single pair of hairs situated on the base of the hypostome. Into the postero-internal angles of the buccal cavity the large cylindrical *salivary ducts* open (s.d.); these are readily recognised by the delicate trachea-like transverse striation of their walls. Opening on the floor of the buccal cavity at its posterior end is the *pharynx*, a longitudinally placed chitinous sac of peculiar shape, which extends backwards into the body-cavity, where it becomes suddenly constricted into the narrow oesophagus. The relation of these parts will be more perfectly understood by reference to the figure showing a schematised longitudinal section through the capitulum (Text Fig. 6, p. 170)<sup>1</sup>.

## II. The Capitulum of the Male.

(Plate XIV, Figs. 3 and 4.)

The capitulum of the male differs from that of the female in details only, but these are sufficient to necessitate a special description. The dorsal ridge of the basis capituli bears at either lateral extremity, a short backwardly directed process (cornua). The porose areas are absent, a few scattered pores of the ordinary type being present. The palps are shorter and wider in proportion to their length and more pointed at the apex. The number of hairs borne on the infra-internal margins of the second article of the palp is usually thirteen, three being carried on the third article, as in the female. The hypostome is narrower in proportion to its length and not so markedly spatulate: the teeth which beset its ventral surface are also more elongated and sharply pointed. The chelicerae agree in their general structure with those of the female, but the digit calls for special attention. The internal article is similar to that of the female; the external article is relatively smaller in comparison with the internal, and only bears three cusps on its external margin. The dorsal process is quite different from

sion of these outlines is misleading, in the fact that they might be taken to represent external or superficial features.

<sup>1</sup> Nuttall, Cooper and Smedley (1905) described the details of the structure and relationship of the buccal cavity and pharynx; it will be considered again in a future paper which will follow in this *Journal*.

that of the female; it consists of a large triangular process, the shape and disposition of which are seen by reference to the figure (Pls. XIV, Fig. 4 and XV, Figs. 5, 6).

### III. The Capitulum of the Nymph.

(Pl. XIV, Figs. 5 and 6.)

The capitulum of the nymph is intermediate in its general appearance between that of the adult and the larva. The salient ridges, seen in the adult on the basis capituli, are fairly well-developed: the anterior portion of the basis capituli is wider in front than behind, and its dorsal surface is produced laterally into large triangular flattened processes with rounded extremities: the postero-lateral angles of the ventral ridge are produced into short rounded backwardly directed processes, which for descriptive purposes we have styled "*cornua*." As in the adult, the basis capituli shows a posterior neck-like constriction immediately behind the dorsal and ventral ridges.

The *hypostome* is less spatulate and is provided on its ventral surface with four rows of denticles, about nine to each row.

The *chelicerae* of the nymph appear to be similar in all respects to those of the female.

The *palps* differ from those of the adult tick. Four articles can be distinguished, but the second and third articles are more or less fused with one another. The first article is very small; is completely concealed within the basis capituli and less readily distinguished than in the adult. The second article is large, and is raised on its ventral, lateral, and dorsal surfaces as a prominent rounded ridge, which forms the pronounced external angle of the palp. The infra-internal margin of this article bears five hairs, flattened and feather-like as in the adult, but wider at their bases and more tapering at their extremities. The third article is conical and forms the extremity of the palp; it is almost completely fused with the second article, the only line of demarcation being found towards the internal margin of the dorsal surface, where a deep indentation in the contour of the edge indicates the commencement of a fissure which is produced round the palp on its dorsal side for a short distance only. A shallow depression runs round the lateral surface of the article, and this, together with the arrangement of the hairs on its proximal side, shows the line of fusion with the second article. The infra-internal margin of the third article carries a single feather-like hair. The fourth article is

similar in form to that of the adult and likewise implanted into an oval fossa on the ventral surface of the third article.

The *porose areas* are absent.

#### IV. The Capitulum of the Larva.

(Pl. XIV, Figs. 7 and 8.)

The capitulum of the larva differs from that of the other stages in the relatively small size of its palps and in the fact that it is shorter in proportion to its width. The basis capituli is divided on its dorsal surface into anterior and posterior portions by a salient ridge, but unlike the later stages no ridge is developed on the ventral surface, and in addition the posterior portion is not so constricted. As in the nymph, a considerable amount of fusion exists between the articles, so much so in fact that the only free article is the fourth, the first, second and third being solidly fused together.

The *hypostome* is smaller than that of the nymph and bears four files of about six denticles.

The *chelicerae* resemble those of the female and nymph in form, all the structures being fully developed as in the adult (Pl. XV, Fig. 3).

The *palps* are very short and conical, strongly curved laterally and deeply concave on their median faces; the lateral angles are not so pronounced as in the nymph. They show a considerable amount of difference from those of the stages already described; the tendency to fusion of the articles as already shown in the case of the nymph reaches a maximum. Article 1 is very small and is exceedingly difficult to define; it is completely hidden within the basis capituli: articles 2 and 3 are the largest, but it is impossible to say with certainty, whether the line of folding of the cuticle (shown by a line in Pl. XIV, Fig. 7) is the line of fusion of the article: article 4 is relatively large and is more terminal in position than in the later stages.

A single feather-like hair is carried on the infra-internal margin of the palp, and in addition to this all the others, with the exception of the stiff bristle-like hairs on article 4, are serrate on one or both sides, as shown in the figures.

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## EXPLANATION OF PLATES.

PLATE XII. *Haemaphysalis punctata*.

Larva. Dorsal and ventral aspects.  $\times 62$ .

Drawn from living specimen by J. Ford.

PLATE XIII. *Haemaphysalis punctata*.

Nymph. Dorsal.  $\times 80$ .

Female, unfed. Dorsal.  $\times 20$ .

Female, gorged. Dorsal.  $\times 5$ . Detail of surface more highly magnified, to left.

Female, gorged. Ventral.  $\times 5$ . Spiracle to right.

Male, unfed. Dorsal.  $\times 15$ .

Male, gorged. Ventral.  $\times 20$ .

Male, unfed. Posterior portion of ventral surface, showing appearance of festoons for comparison with those of the gorged male.  $\times 20$ .

Drawn from living specimens by E. Wilson.

PLATE XIV. *Haemaphysalis punctata*.

Fig. 1. Capitulum of Female. Dorsal aspect.  $\times 85$ .

Fig. 2. " of Female. Ventral aspect.  $\times 25$ .

Fig. 3. " of Male. Dorsal aspect.  $\times 110$ .

Fig. 4. " of Male. Median portion, with left palp. Ventral aspect.  $\times 125$ .

Fig. 5. " of Nymph. Dorsal aspect.  $\times 220$ .

Fig. 6. " of Nymph. Ventral aspect.  $\times 220$ .

Fig. 7. " of Larva. Dorsal aspect.  $\times 400$ .

Fig. 8. " of Larva. Ventral aspect.  $\times 400$ .

PLATE XV. *Haemaphysalis punctata*.

Fig. 1. Female. Tactile hairs on infra-internal margin of palp.  $\times 250$ .

Fig. 2. Male. Ventral surface, showing details of external structures.  $\times 80$ .

Fig. 3. Larva. Chelicerae, from ventral surface.  $\times 1000$ .

Fig. 4. Female. Right chelicera, from ventral surface; sheath removed.  $\times 250$ .

Fig. 5. Male. Left chelicera, from ventral surface.  $\times 250$ .

Fig. 6. Male. Right chelicera, lateral aspect; sheath removed.  $\times 250$ .

PLATE XVI. *Haemaphysalis punctata*.

Female. Transverse sections through the capitulum showing the relationships of the chitinous structures.  $\times 125$ .

Fig. 1. Transverse section at level of digits of chelicerae (chelicera retracted).

Fig. 2. " " " " " distal third of hypostome.

Fig. 3. " " " " " proximal third of hypostome.

Fig. 4. " " " " " base of hypostome.

Fig. 5. " " " " " insertion of palps.

Fig. 6. " " " " " base of palps.

Fig. 7. " " " " " anterior margin of porose areas.

Fig. 8. " " " " " centre of porose areas.

Fig. 9. " " " " " posterior margin of porose areas.





*dorsal aspect*



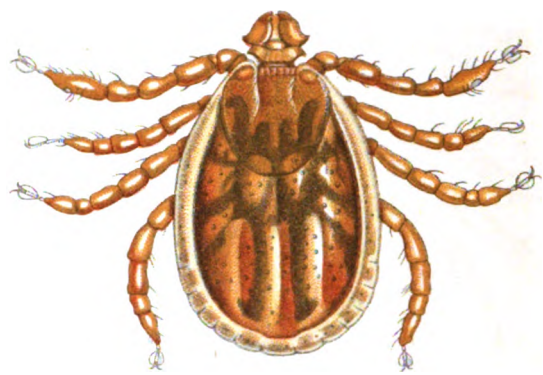
*ventral aspect*

E Wilson, Cambridge.

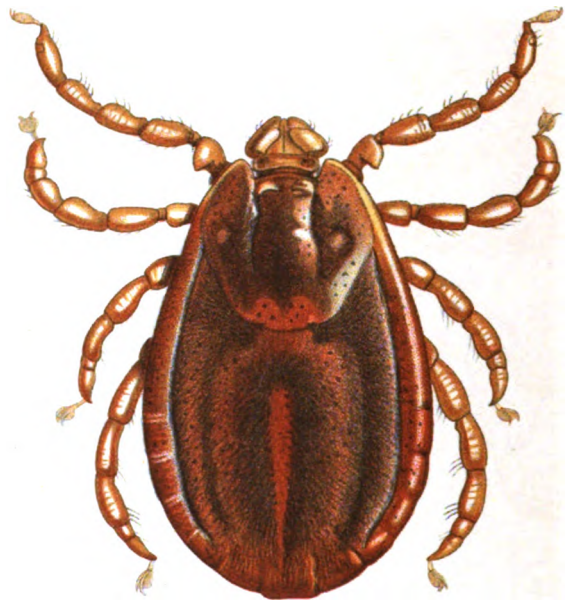
HAEMAPHYSALIS PUNCTATA Can & Fan.  
THE LARVA.



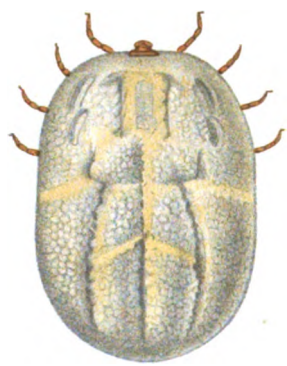




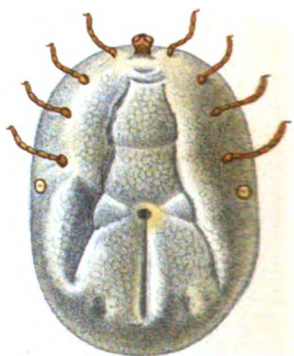
Nymph



Female (*unfed*)



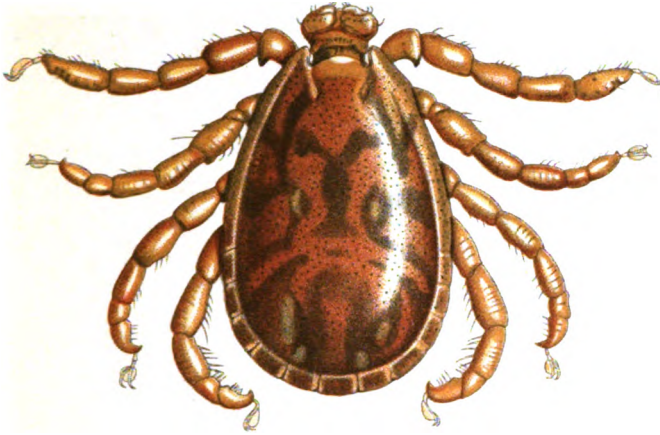
*dorsal aspect*



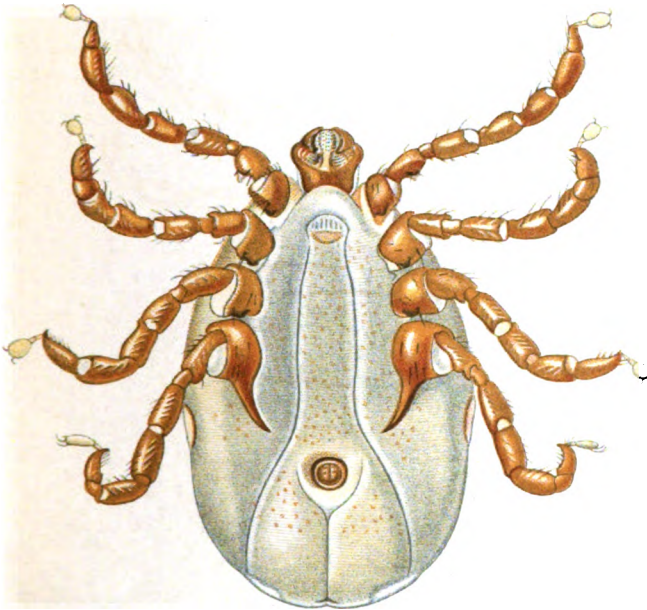
*ventral aspect*

Female (*replete*)

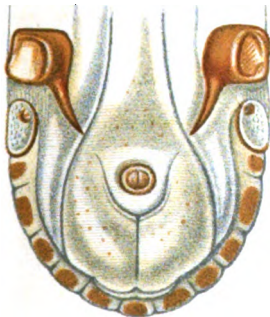
HAEMAPHYSAL



Male



Male (*replete*)



Male (*unfed*)

E. Wilson, del et lith. Cambridge





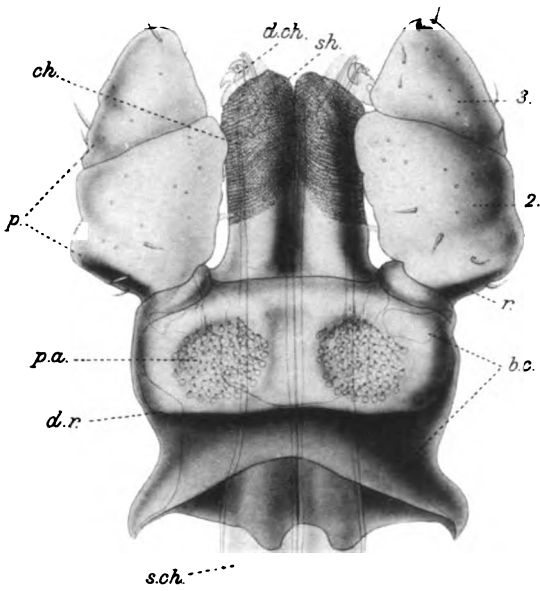


Fig.1.

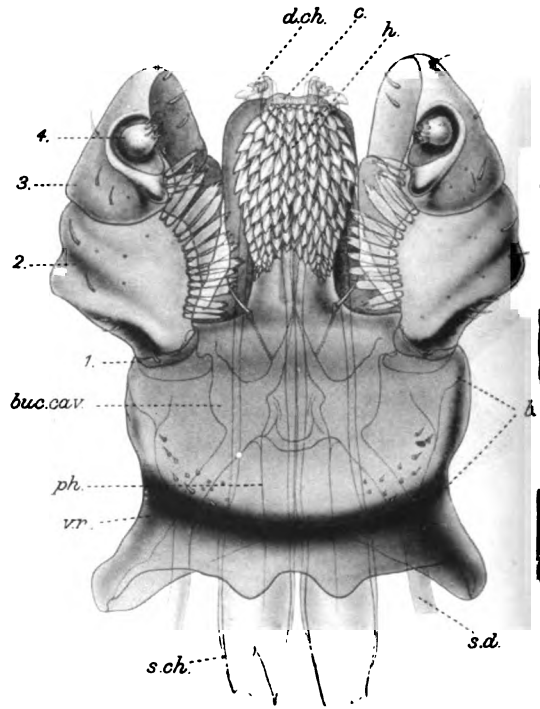
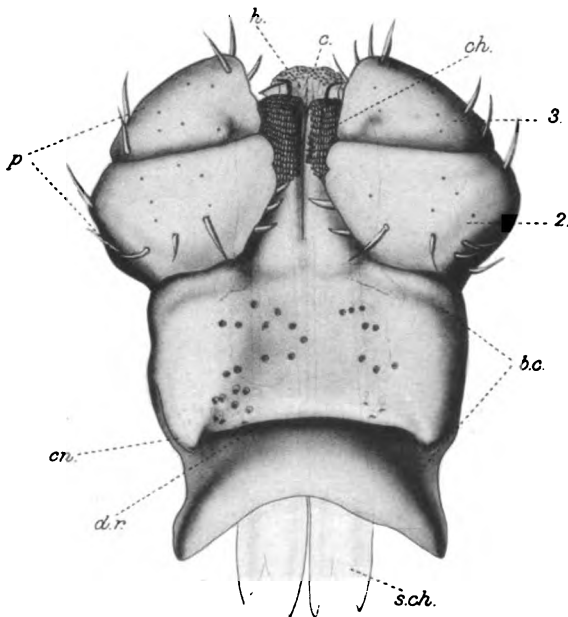


Fig.2.



L.E.R. and J.Ford. del

Fig.3.

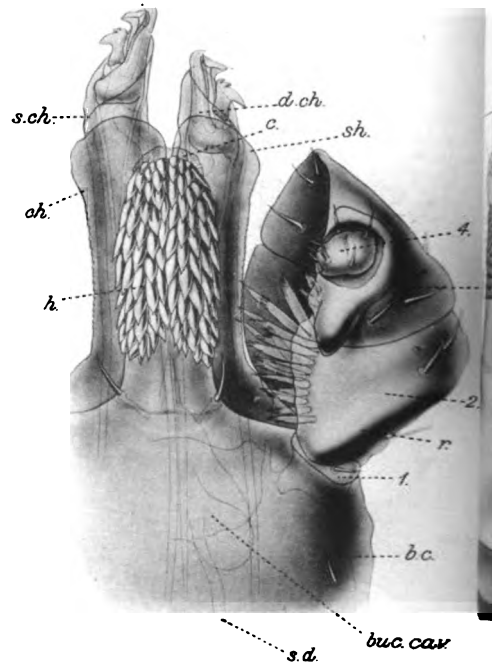


Fig.4.



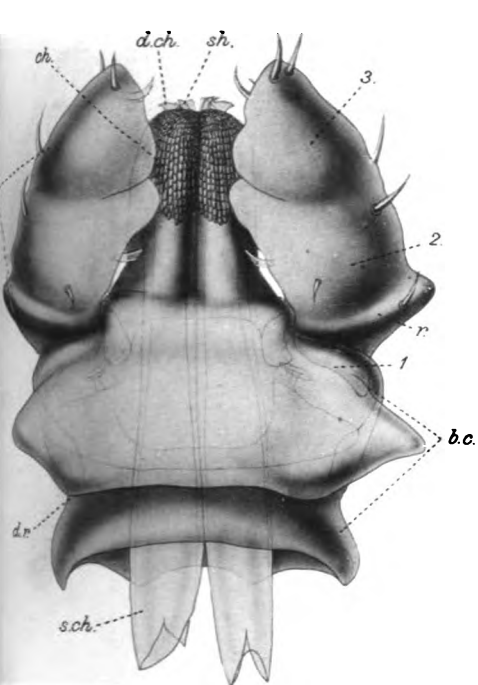


Fig. 5.

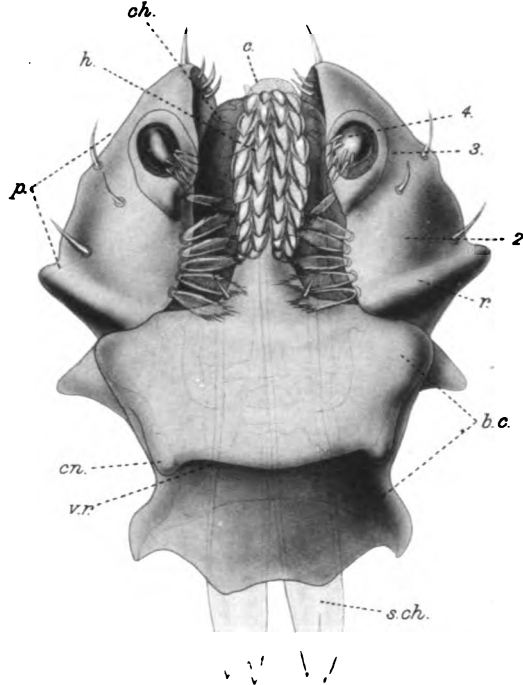


Fig. 6.

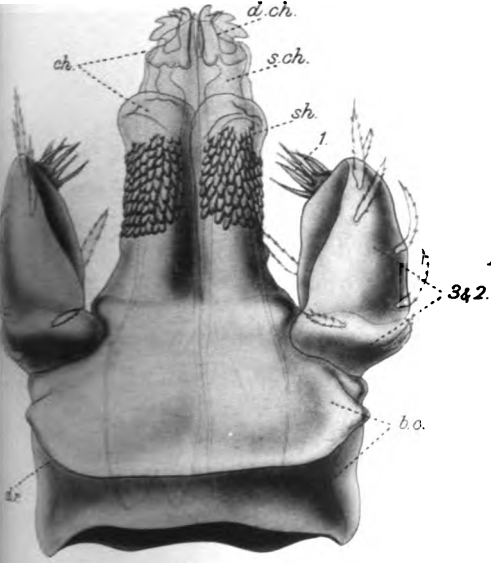


Fig. 7.

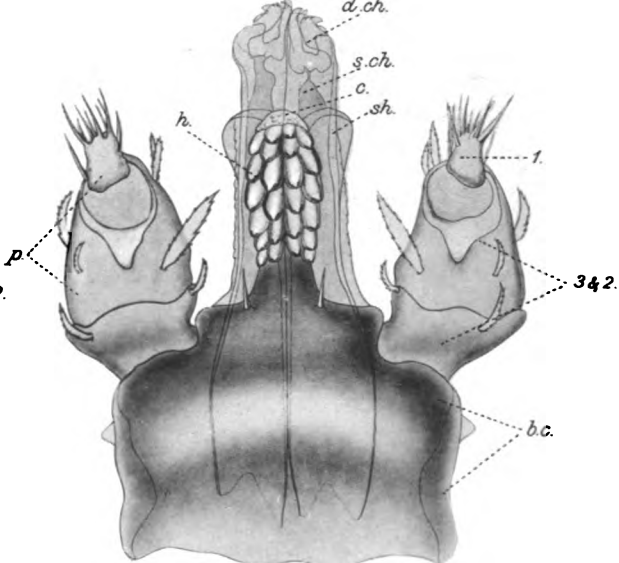


Fig. 8.

Morgan & Kidd, Richmond, Collotype.



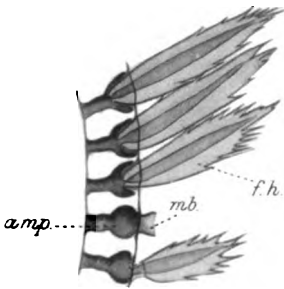


Fig. 1.

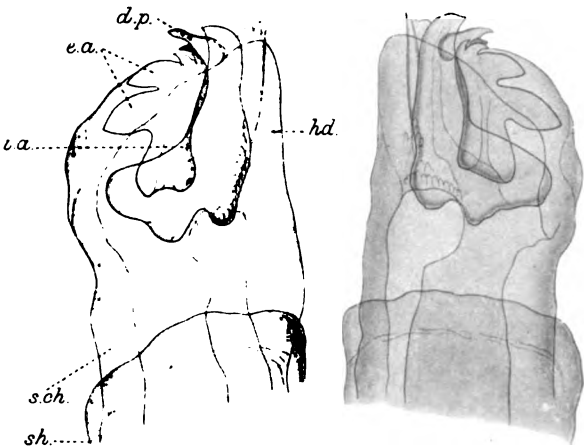


Fig. 3.

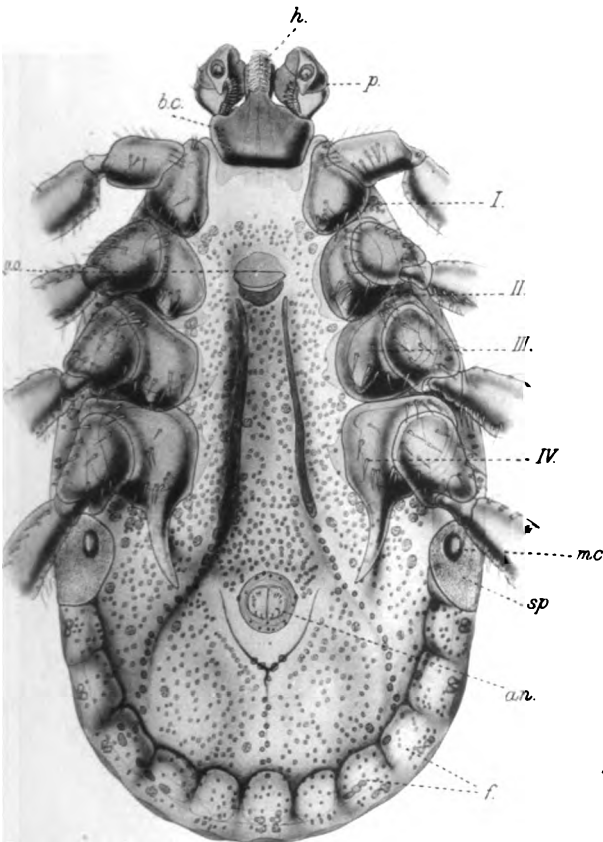


Fig. 2.

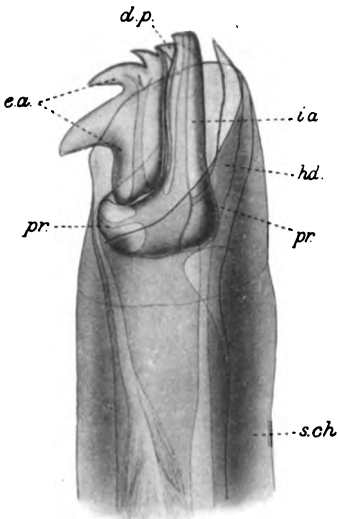


Fig. 4.

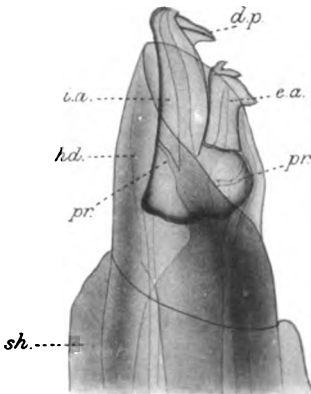


Fig. 5.

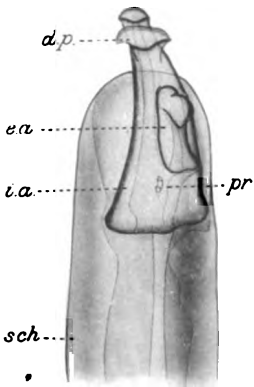


Fig. 6.



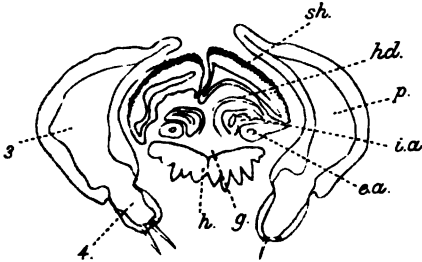


Fig. 1.

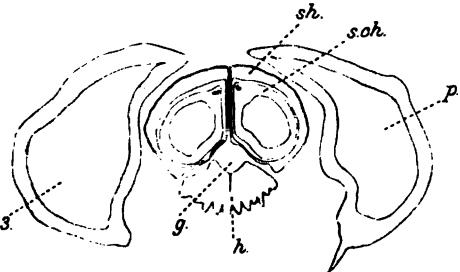


Fig. 2.

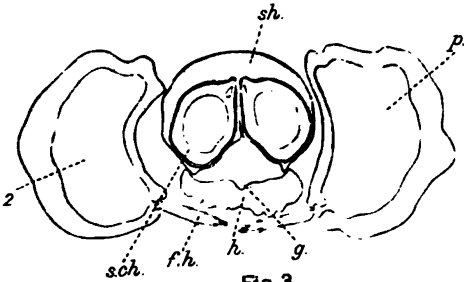


Fig. 3.

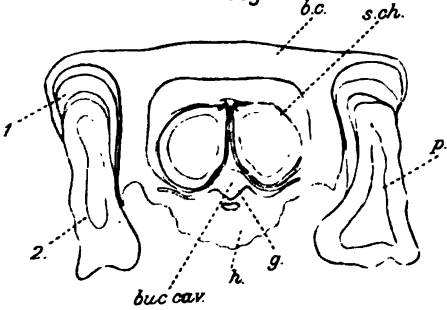


Fig. 4.

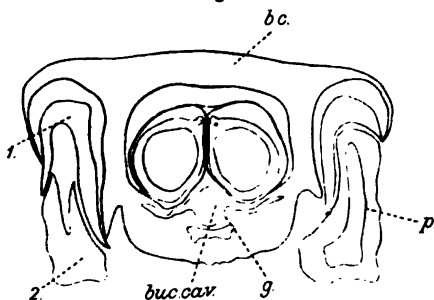


Fig. 5.

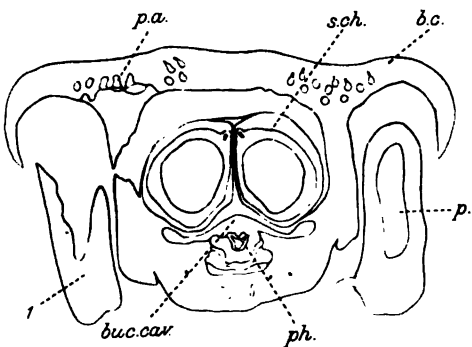


Fig. 6.

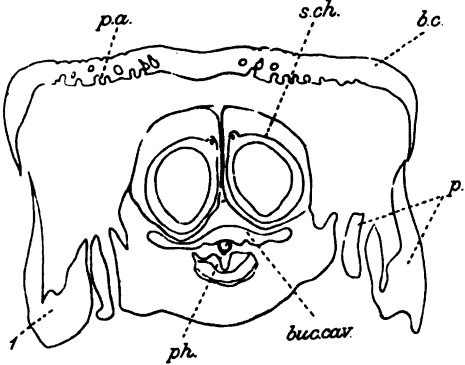


Fig. 7.

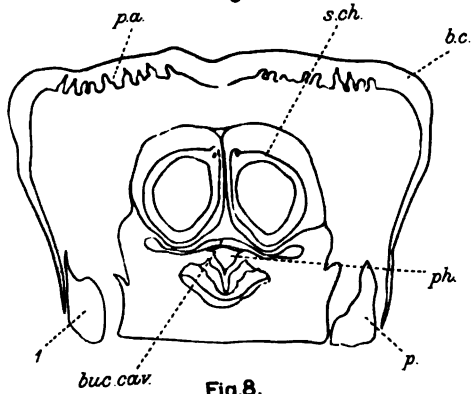


Fig. 8.

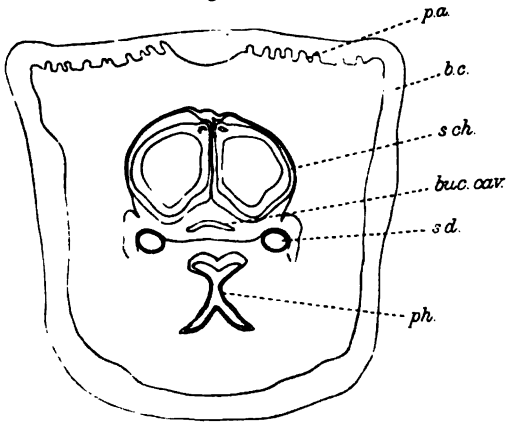


Fig. 9.



INDEX TO LETTERING ON PLATES XIV—XVI.

<i>amp.</i>	ampulla at base of hair.
<i>an.</i>	anus.
<i>b. c.</i>	basis capituli.
<i>buc. cav.</i>	buccal cavity.
<i>c.</i>	corona.
<i>ch.</i>	chelicera.
<i>cn.</i>	cornu.
<i>d. ch.</i>	digit of chelicera:
<i>d. p.</i>	dorsal process.
<i>d. r.</i>	dorsal ridge.
<i>e. a.</i>	external article of digit.
<i>f.</i>	festoons.
<i>f. h.</i>	feather-hair on infra-internal margin of palp.
<i>g.</i>	gutter on dorsal surface of hypostome.
<i>g. o.</i>	genital orifice.
<i>h.</i>	hypostome.
<i>hd.</i>	hood surrounding digit.
<i>i. a.</i>	internal article of digit.
<i>mb.</i>	flexible membranous portion of feather-hair.
<i>p.</i>	palp.
<i>p. a.</i>	porose area.
<i>ph.</i>	pharynx.
<i>pr.</i>	pores on article of digit.
<i>r.</i>	ridge on article 2 of palp.
<i>s. d.</i>	salivary duct.
<i>sh.</i>	sheath of chelicera.
<i>s. ch.</i>	shaft of chelicera.
<i>v. r.</i>	ventral ridge.
1. 2. 3. 4.	articles of palps.
I. II. III. IV.	coxae.

## HIRUDINEA AS HUMAN PARASITES IN PALESTINE.

By E. W. G. MASTERMAN, F.R.C.S., D.P.H. Camb.

LEECHES are common in the fountains and pools of Palestine, particularly in the northern parts, known to us familiarly as 'Galilee,' and further north in the district of the Lebanon. In the later summer and autumn months they are so plentiful in places that almost every horse and mule passing through these parts has a bleeding mouth. Under such conditions it is not wonderful that human beings from time to time are attacked. The domestic supplies of water are usually protected by the water-carrier's custom of straining it through a piece of fine muslin as he or she fills the pitcher. In some parts of the land the water at the source is kept free from leeches by means of fish; at Deishun, for example, a village in 'Upper Galilee' inhabited by Algerian settlers, the large stone tank into which the spring runs is full of a special fish—*Capoëta fratercula*, a kind of carp—which is there bred for the purpose and which is treasured by the inhabitants with superstitious reverence. Many of the springs in the mountains of Galilee and in the Lebanon are widely known for the multitude of leeches which lurk in their waters, but a thirsty traveller seldom has self-control enough to restrain himself from drinking, and when he does so, particularly at dusk or in the night, he is very likely to suck one in. It is stated in Allbutt and Rolleston's *System of Medicine* (2nd ed. Vol. II. Part II. p. 959) that the leech "remains in the stomach for a time and then begins to wander." I have had a long experience of leeches as human parasites but have never seen nor heard of a case like this. The leech in every case I have met has attached itself to the mouth, throat, etc. in the process of swallowing, and I am convinced that once the leech reaches the stomach it is killed. I have never heard of any after symptoms from cases where to my knowledge a leech has been completely swallowed. Many patients can describe the actual moment



(during deglutition) when the leech seizes on the throat. One would suppose from the great frequency of leeches in the mouths of horses, etc., that they must sometimes find a temporary resting place there in the human subject; such cases do not however come into the hands of a medical man—the patient or his friends readily removes such an intruder. In the cases, some three dozen or so, which I have had under my own observation the leech has been attached to the epiglottis, the pharynx, the nasal cavities, or—most common of all—the larynx. The frequency of the last situation in my clinical experience is doubtless due to the inability of the *fellahin* to do anything themselves for the relief of such cases.

The characteristic symptom produced by *Hirudinea* is the occurrence of constant small hæmorrhages from the mouth or nose. Last summer in Safed I had a baby of about seven months brought to me with a history of almost continuous slight epistaxis from the left nostril of a week's duration. The child had been brought a considerable distance for treatment. On examining the nostril I noticed something blackish a little way up, and introducing a pair of fine sinus forceps I had the satisfaction of drawing out a slender leech about  $1\frac{1}{2}$  ins. long. The symptoms were at once relieved. The first case I had, on September 16, 1893, also at Safed, was remarkable. The patient, a girl of 12, came complaining of constant small hæmorrhages from the mouth for three days; there was no dyspnoea nor aphonia. I put my right index finger well to the back of the tongue to see if I could feel anything abnormal in the neighbourhood of the epiglottis, and when I drew it forth was astonished to find a small leech looped up upon my finger-nail. These are mild cases. When the leech lies deeper the symptoms are much more distressing. The hæmorrhage, though never great in quantity at any one time, may, when prolonged, be very serious and even fatal. I know of two cases where the patient actually died from this. One, which occurred shortly before I came to Palestine, was narrated to me by Dr Torrance of Tiberias. The man came under medical treatment already reduced to extreme anaemia by prolonged hæmorrhage; all efforts failed to reach the leech and it was only after complete eversion of the patient that it was at last extruded, but the patient never recovered the loss of blood. The second case is that of a young fellah girl who was brought to Safed last summer for the removal of a leech which she had harboured in her throat for some weeks. She just reached Safed before she died as a result of anaemia.

The hæmorrhagic discharge is pure blood, bright in colour and,

when the leech is in the pharynx or larynx, it is 'hawked up' almost continuously. As a rule, and of course in all laryngeal cases, there is considerable irritation about the larynx resulting in a short irritating cough, dyspnoea, varying in intensity from time to time, and sometimes considerable cyanosis from impeded respiration. The patient's expression is one of considerable distress, a great contrast to that following upon the instant relief afforded by the removal of the parasite. In laryngeal cases there is often complete loss of voice, the patient only speaking in a whisper and then with difficulty. On examination with a tongue depressor the leech is sometimes revealed, lying at the back of the pharynx; in such a situation it cannot be mistaken as its black, rounded, annulated, shiny outline at once catches the eye. More commonly it is necessary to use the laryngoscope, and in a considerable proportion of cases the leech will be found attached close to one of the vocal cords. Its head attachment is sometimes just inside or just outside the cord, and it flops in and out of the aperture with the respiration. In such cases the wonder is not that the patient suffers from dyspnoea, but that he can breath at all. The upper larynx and the pharynx are largely bespattered with fresh blood and in the process of examination the patient usually coughs out a good deal of it. Why it is that a leech, which outside the body takes its fill and then drops off or is easily removed, continues when inside the mouth and throat to keep its hold, though apparently attacking different spots—leaving the old points to bleed—is a question I must leave to zoologists, but it is certainly the case, and in my experience in such a situation it seldom voluntarily quits its hold. According to the *System of Medicine* quoted above this species of *Hirudinea* is *Limnatis nilotica*.

As regards the treatment, the natives of Palestine are accustomed if the worm is within their reach to transfix it with a sharp thorn from the *Sidr* tree: I have seen muleteers use a packing needle to extract a leech from a mule's mouth. When the parasite is beyond reach of this process they use what they call *es salata*, that is the thick deposit which collects in their tobacco pipes. This they smear on the end of a fragment of wood and they say that if this touches the leech it is poisoned and leaves its hold. The cases which came to the European practitioner are almost always those where the skill of the amateur has failed and for these such simple means are useless. In my experience too the 'spraying with salt solution' mentioned in Allbutt and Rolleston's *System* has proved of little use. The two means I have found most successful are either mechanically seizing the worm by means of suitable forceps or paralysing it with cocaine.

In a certain proportion of cases it is possible to seize the leech. With pharyngeal cases this should be the rule and perhaps with throat specialists it might also be the case with laryngeal cases. For the ordinary surgeon, though it is simple enough to watch the worm through laryngoscope mirror, it is very difficult to seize the writhing, slippery creature amidst the spasmodic contractions of the larynx and the frequent coughing. Spraying with cocaine is a great help, but I must admit that in mechanically removing the leech I have several times had failures. The second method, which I have never yet known to fail, I owe to the suggestion of Dr Mackinnon of Damascus—this is the application of a strong solution of cocaine to the parasite. It is not enough to spray in cocaine or paint it indiscriminately. For sure success a small pledget of cotton-wool, saturated with a 30 % cocaine solution, should be actually brought in contact with the parasite by carefully directed movements guided by the help of the laryngoscopic mirror. The strong cocaine solution, if it reaches the surface of the leech undiluted always produces in a few minutes—not, in my experience, instantaneously—a paralysing effect, causing it to relinquish its hold. My last three cases, all treated in this way, were speedily successful. In the case of one of these patients the man apparently swallowed the leech for he had no more symptoms from a few minutes after treatment; in the second case the leech was suddenly coughed into the mouth about five minutes after the cocaine application and I removed it from the mouth with my finger. The last case was one I found in our Hospital in Jerusalem immediately on my return from Safed. The man had for nearly a week been 'spitting blood,' and when I was called to see him a spittoon full of practically pure blood was lying on the locker by his side and every few minutes he was adding more: his lips were cyanosed; he was unable to speak above a whisper and every few seconds he had a short cough. I sprayed cocaine solution upon the fauces and the laryngeal mirror at once revealed a large leech attached close to one of the vocal cords. After several vain efforts to seize it with curved forceps I applied cocaine as described and then made the man lie prone upon his bed with his head hanging over the edge. After a few minutes he coughed up the leech on to the floor. I have heard it stated that there is a danger that the parasite on leaving its hold may fall downwards and lodge on one of the bronchi, then die and set up septic changes. I have never seen such a case, but such a danger is doubtless obviated by making the patient assume the position I have described when the cocaine is commencing to have its effect.

NOTE ON A GNATHOBDELLID LEECH [*LIMNATIS* SP. ?]  
FROM ANGOLA.

By W. A. HARDING, B.A.

I OWE to Prof. G. H. F. Nuttall and Mr A. E. Shipley the opportunity of examining three specimens of a West African leech sent by Dr C. Wellman from Benguella, Angola. They were described as land leeches 'taken four or five miles from any water' and were preserved in alcohol. A brown, vermiform object protruded from the mouth of the largest specimen.

Transverse sections of this object revealed muscular tissue but gave no further indications of its nature. Many leeches [e.g., *Trocheta*; *Aulastoma*, Moq. Tand.] are known to leave the water voluntarily in search of food such as worms and molluscs, and it may be assumed with some certainty that this specimen possessed similar habits and had been killed in the act of devouring its prey.

*Diagnostic characters.*

The body resembles *Hirudo* in form, except that, anteriorly, it tapers rather more rapidly. The following measurements were taken :

Largest specimen : 51·4 mm. long and 9·7 mm. in width.

Smallest specimen : 26 mm. long and 5·60 mm. in width.

The acetabulum is circular and of moderate size.

The colour is brownish-grey without any trace of pattern or coloured spots, rather paler on the ventral surface. There is an ill-defined, median, dull yellow tract on the dorsal side, following the course of the alimentary canal.

Eyes 10; arranged, as in *Hirudo*, on the 1st, 2nd, 3rd, 5th and 8th annuli [Fig. 2]. The first three pairs are distinct, the fourth pair less apparent, the fifth pair almost imperceptible. They are best seen on the smallest specimen.

Annuli 102; counting from the first pair of eyes to the acetabulum. The 5th and 6th [the buccal annuli of Whitman] are united ventrally to form the first ventral ring; the 6th and 7th are united ventrally to form the second ventral ring [Fig. 1]. The annuli have a shallow, median wrinkle, parallel to their sides, which

is sometimes split up into subsidiary wrinkles. This feature may be due to contraction in alcohol.

The large and prominent anus lies in the 102nd annulus, causing it to become double [Fig. 4].

The genital apertures are five rings apart, small and difficult to see. The male orifice lies between the 31st and 32nd annuli; the rather larger female orifice lies between the 36th and 37th annuli.

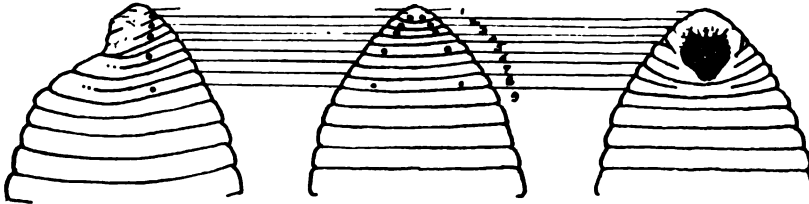


Fig. 1. Side view.

Fig. 2. Dorsal.

Fig. 3. Ventral.

Diagrams showing eyes and anterior rings.

The horizontal lines connecting the figures define the annulations which are numbered 1—9 in the middle figure (annulation 1, indicated by a broken line, was indistinct).

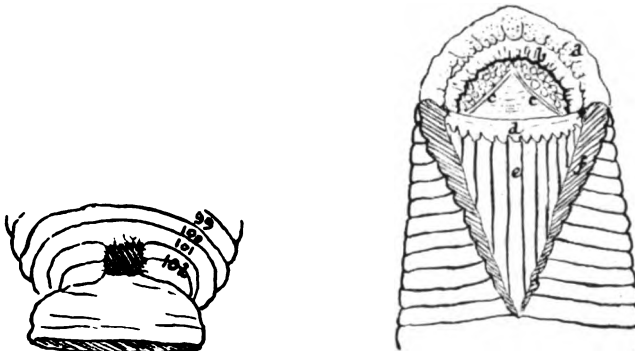


Fig. 4.

Fig. 5.

Fig. 4. Dorsal aspect of posterior rings and acetabulum.

Fig. 5. Diagram of ventral surface of anterior sucker.

- a. Outer lip.
- b. Its inner, crenulate border.
- c.c. The diverging ridges.
- d. Edge of mouth with frilled membrane.
- e. Interior of pharynx.
- f. Cut edge of body, opened by a median ventral incision.

The general characteristics of the leech indicate that the complete somite is composed of five annuli. As no segmental papillae or nephridial pores can be detected [owing to immersion in alcohol?] it is impossible to determine this point with certainty from external examination.

The crop, which was not fully investigated posteriorly, has paired caeca like *Hirudo*.

The blood is red.

The pharynx is long and plicated as in *Aulastoma*, Moquin Tandon. In the largest specimen these plications or folds are indistinct; in the second specimen twelve, equal, longitudinal plications were clearly seen.

The ventral side of the anterior sucker is bounded anteriorly by a deeply wrinkled lip with an inner crenulate border. From a median point slightly behind this border, two ridges diverge posteriorly, dividing the hollow, ventral surface of the sucker into three depressions and forming a triangle with the edge of the mouth, which appears as a transverse ridge in individuals laid open by a median, ventral incision as shown in Fig. 5. The depressions within and without this triangle vary considerably in form according to the amount of stretching to which the individual is subjected, a fact which may account, to some extent, for the conflicting descriptions of similar features in other leeches.

The ridge formed by the edge of the mouth is extended posteriorly into a frilled membrane overhanging the ends of the pharyngeal plications and beneath it the maxillae, if present, should be found.

Although the above diagnostic characters are not complete, it is evident that Dr Wellman's leech is one of the *Gnathobdellidae*, a group which Dr R. Blanchard divides into the two sub-families *Hirudininae* and *Haemadipsinae*.

The presence of a ring between the third and fourth pairs of eyes is sufficient to separate this leech from the *Haemadipsinae* or true land leeches, and it therefore must be placed in the first sub-family.

The same authority assigns to the *Hirudininae* the following genera, viz., *Hirudo*, *Haemopsis* [= *Aulastoma*, Moquin Tandon], *Limnatis*, *Hirudinaria*, *Macrobdella*, *Whitmania* and *Limnobdella*.

This leech differs, amongst other characters, from *Hirudo* in the absence of the three powerful jaws; from *Haemopsis* [= *Aulastoma*, M.T.] in the form of the crop; from *Hirudinaria* and *Macrobdella* in the number of rings separating the genital apertures. I am unable to speak positively with regard to *Limnobdella* and *Whitmania* as I have been unable to analyse somites VI and XXIII owing to the absence of segmental papillae. Taking everything into consideration I venture to place Dr Wellman's leech in the genus *Limnatis* which it appears to resemble most closely. In particular, Dr Blanchard's description of the anterior sucker of *Limnatis* as "creusée d'un sillon en dessous" and Moquin-Tandon's description of the same as "profondément creusée en dessous d'un canal en triangle" seem to agree with the characters shown in Fig. 5.

[N.B. It is to be noted that Professor Blanchard agrees with Leuckhart, Whitman and Apáthy in rejecting *Haemopsis*, Moquin Tandon, 1846, as unworthy of generic rank.

He substitutes *Haemopsis*, Savigny, 1817 for *Aulastoma*, Moquin Tandon, 1826; and *Aulastoma gulo*, Moquin Tandon, 1846, becomes *Haemopsis sanguisuga*, Bergmann, 1757.]

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NOTE ON *CYSTIDICOLA FARIONIS* FISCHER. A THREAD-  
WORM PARASITIC IN THE SWIM-BLADDER OF A TROUT.

BY A. E. SHIPLEY, M.A., Hon. D.Sc. (PRINCETON), F.R.S.,  
*Fellow and Tutor of Christ's College, Cambridge.*

*CYSTIDICOLA FARIONIS.* G. Fischer, 1798.

Synonyms: *Fissula farionis.* Bosc, 1802.

*Fissula cystidicola.* Lamarck, 1801.

*Ophiostoma cystidicola.* Rudolphi, 1809.

*Spiroptera cystidicola.* Rudolphi, 1819.

*Dispharagus cystidicola.* Dujardin, 1845.

*Ancyracanthus cystidicola.* Schneider, 1860.

*Ancyracanthus cystidicola.* von Linstow, 1878.

SOME hundred and ten years ago, Dr Gotthelf Fischer, in a communication dated from Vienna, described a Nematode living in the swim-bladder of the trout. He named this worm *Cystidicola farionis* and his description is accompanied by some rough cuts. Bosc (1802) re-described the same Nematode under the name *Fissula farionis*. The same worm was again described by Rudolphi (1819) under the name *Spiroptera cystidicola*. Dujardin (1845) mentions it under the name *Dispharagus cystidicola*. Later it was removed by Schneider (1860) from the genus *Spiroptera* and placed in Diesing's genus *Ancyracanthus*. von Linstow uses this generic name "with a difference"; he spells it *Ancyracanthus*.

Ramsay Wright (1879) records the species from *Salmo siscowet* Agassiz. It is widely spread on the continent of Europe and is now—I think for the first time—recorded in England.

Mr R. T. Leiper of the London School of Tropical Medicine, who kindly examined some specimens of these round worms which I had recognized as belonging to Schneider's species *A. cystidicola*, has pointed out that the worms were originally described and figured by G. Fischer and that his name *Cystidicola farionis* still stands. Mr Leiper has written a note on the anatomy of the worm and his description follows (see p. 193).



Certain trout found in the streams on the estate of the Hon. Sydney Holland, Royston, Herts., have been dying off in considerable numbers during the spawning season for the last year or two. Some of these were sent to Cambridge this spring, and, on dissection, it was found that the cavity of the swim-bladder was infested with Nematode worms of the species described under the name *A. cystidicola* by Schneider, now re-called *C. farionis*.

In his original description Fischer draws attention to the curious atmosphere in which the worm lives. He describes the gas in the swim-bladder of the trout as being almost pure nitrogen with a little CO<sub>2</sub>. Whether a more recent analysis of the gas from this fish's swim-bladder has been made I do not know but as a rule in fresh-water fishes, to quote Günther, the gas "consists of nitrogen with a very small quantity of oxygen and a trace of carbonic acid." The proportion of oxygen is usually greater in sea-fishes and it also varies in the same fish at different seasons. Bunge (1890) has shown that *Ascaris mystax* from the intestine of the cat can live for four or five days in an atmosphere quite free from oxygen and that in a similar medium *A. acus* from the pike will live and move for from four to six days. Probably the traces of oxygen in the trout's swim-bladder are sufficient to supply the meagre wants of *C. farionis*.

The parasites are stated to be more numerous in the winter: in the late spring they seem to average 8 to 18 or so in each fish. They have also been met with in the oesophagus and probably enter the swim-bladder through the "ductus pneumaticus."

The question whether the presence of these parasites is injurious to the fish is a debateable one. They are usually regarded as harmless. Certainly many of Mr Holland's trout have been dying but the worms were found in the trout before the mortality set in, and in those fish we examined at Cambridge they were at least as numerous in fish reported as being healthy as in those fish that had died. On the other hand all the dying fish appear to suffer from some derangement of the swim-bladder; they swim always on the surface on the water and die with their heads downwards and the body almost if not quite perpendicular to the surface of the water. They die, as Mr Holland tells me, not only in deep water but occasionally in the shallow water of the spawning beds. This year however during the period of greatest mortality when 6 or 7 trout were dying a day until some 50 out of 200 were dead, they never tried to work up stream to the spawning beds but died in the deep water. The fish seemed to have difficulty about shedding their ova and it may be

that this difficulty is connected with the cause of the mortality, but males died with the same symptoms as females, though not in such large numbers. Since the spawning season finished this year there have been no deaths. Curiously, too, the rainbow trout are alone affected and not the brown trout.

I am indebted to Mr Holland not only for the Nematodes but for many of the facts mentioned in this note.

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## NOTE ON THE ANATOMY OF *CYSTIDICOLA FARIONIS*.

By R. T. LEIPER, M.B., F.Z.S.

*Helminthologist to the London School of Tropical Medicine.*

THE nematode worms discovered by Mr Shipley in the swim-bladder of Trout belong to the species *Cystidicola farionis*, first described by Fischer, and considered by him to represent a new and distinct type in nematode structure for which he proposed the generic name *Cystidicola*. Later writers refused to accept the new genus and included it in various other groups (see Synonymy, p. 190). In accordance with an old nomenclatural practice the proposed generic name was adopted as the specific name in place of that originally given, thus *Cystidicola farionis* became, for Rudolphi, *Spiroptera cystidicola*. From a detailed examination of the various characters of *C. farionis* I have come to the conclusion that Fischer was correct in creating a new genus for the new forms, and I therefore propose the reinstatement of the abandoned name *Cystidicola*. It is also in accordance with modern nomenclatural ruling that the original specific name *farionis* should be adopted in place of *cystidicola*.

### *The anatomy of C. farionis.*

The body is very slender and translucent, it tapers gradually from the middle third to both ends of the body. In preserved specimens the male is coiled posteriorly in a spiral manner and measures about 14 mm. in length. The female remains perfectly straight, measuring 22—26 mm.  $\times$  3 mm. The cuticle is thin and without transverse striation. At the posterior end of the male it expands to form a narrow ala on either side supported by the genital papillae. In the female its uniformity is disturbed only by a tiny pair of papillae near the tip of the tail.

The alimentary canal shows the usual differentiation into oesophagus, chyle intestine and rectum, the first-named structure presenting characters that associate *Cystidicola* with the other genera of the family Spiruridae. The mouth is a simple spherical orifice surmounted by two hook-like teeth that in shape recall the "external tooth" of *Physaloptera* species. It gives access to a deep cylindrical mouth capsule with thick

chitinous wall measuring  $\cdot 1 \text{ mm.} \times \cdot 01 \text{ mm.}$  The oesophagus is exceedingly long and narrow; it measures about  $2\cdot 7 \text{ mm.} \times \cdot 5 \text{ mm.}$ ; the anterior fifth is narrower than the succeeding portion and does not contain muscular fibres. The nerve ring encircles this slender part at its middle, i.e. about  $\cdot 3 \text{ mm.}$  from the anterior end of the body. The male genital system presents the following features: the testicular tube extends forwards without a single convolution from the cloaca along the ventral surface of the chyle intestine to end blindly at the junction of the anterior and posterior halves of the body. There are two spicules, unequal in size and thickness; the long slender spicule measures  $\cdot 75 \text{ mm.}$ , the short one  $\cdot 16 \times \cdot 025 \text{ mm.}$  The cloaca opens  $\cdot 2 \text{ mm.}$  from the tip of the tail and is enclosed on either side by a narrow cuticular ala extending from the end of the body along each lateral edge to a similar distance in front of the cloacal orifice. Within the alae are seen a double series of nipple-shaped papillae, there being nine pairs of prae-anal papillae and five single post-anal papillae in each ala. The components of the most anterior pair of prae-anals are considerably separated, whilst those of the other pairs lie close together, one slightly internal to the other.

The female genital system opens by a very short vagina in the centre of the ventral surface, midway between the head and tail.

From the vulva the vagina passes backwards, not forwards as stated by Schneider, for a distance equal to the diameter of the worm at that level and there divides into two uterine tubules, one of which continues directly backwards for a distance equal to a fourth of the body-length, doubling upon itself once in this distance in a coil of half that length and is then continued as an ovarian tubule. The other uterine tubule turns abruptly forwards from the vagina and describes a very similar course in the anterior region of the perivisceral cavity. Both ovarian tubules make two or three short coils. Those of the anterior tube only slightly overreach the junction of the oesophagus with the intestine, those of the posterior one do not extend so far as to encroach upon the posterior sixth of the body.

The mature female is ovoviparous, the eggs have thick shells with a curious tuft of exceedingly delicate filaments, two or three in number, attached to a small cuticular knob at either pole. The eggs measure  $\cdot 05 \times \cdot 025 \text{ mm.}$  and the shell is  $\cdot 005 \text{ mm.}$  thick. The only other nematode in which such filaments have been noted, to my knowledge, is *Ascarophis morrhuæ*, van Beneden (1870)<sup>1</sup>, in which species there are cuticular appendages at one pole only.

<sup>1</sup> van Beneden (1870), *Les poissons des côtes de Belgique*, Plate III. fig. 11.

## BILHARZIOSIS IN SOUTH AFRICA.

BY G. A. TURNER, M.B., CH.B., D.P.H. ABERDEEN.

*Medical Officer to the Witwatersrand Native Labour Association, Ltd.*

A FEW years ago it was believed that there was only one form of trematode responsible for the pathological conditions included in the term Bilharziosis, namely a worm, known as *Schistosoma haematobium*, which has had numerous other synonyms, but as I am dealing with the subject purely from a South African point of view, I need merely mention that given it by Dr John Harley, namely *Distoma capense*.

It is now recognised that there are several varieties of Bilharzial parasites.

In South Africa, I have only found evidences of *Schistosomum haematobium* in both its vesical and intestinal manifestations. *Schistosomum japonicum* has not come under my notice.

In this country the disease caused by these parasites is an important one, especially among the native races; far more important than I think is generally believed. So many medical men, born in this country, have in their youth passed blood-stained urine, or have been accustomed to hear of friends doing so, without suffering apparent serious consequences, that they are liable to look upon it as an affection of the bladder only, and to attach little importance to it, forgetting possibly that it may attack other organs, frequently with serious consequences.

In many parts of the Cape Colony and the Transvaal, a large number of the European youths contract the disease. School boys in these parts have competitions as to who can pass a urine of the deepest colour, and the boy most seriously infected is considered more fortunate than his fellows.

Doubtless most of the European boys in Cape Colony, Natal and the Transvaal get rid of all symptoms at about 20 years of age, the disease dying out, possibly because the patient is removed to some

place where re-infection cannot occur. But among the natives living in the tropical or semi-tropical parts, the complaint does not take such a favourable course.

There is probably no disease which causes the native inhabitants of the Province of Mozambique so much worry and anxiety as Bilharziosis. They attribute every evil under the sun to it: impotency of men and sterility in women, are often considered the result of infection. I must admit there seems some justification for the belief. There cannot be any doubt that the disease in any case has a debilitating effect on the native's constitution, probably rendering him more subject to tuberculosis and other diseases when he leaves his kraal to seek work in a colder climate. When the parasite attacks the intestine the result is often fatal.

#### *Geographical Distribution in South Africa.*

In 1851, Bilharz and Griesinger discovered that the haematuria in Egypt was due to the presence of a trematode worm and its ova in various parts of the body. In 1864, Dr John Harley announced the discovery of the parasite in Port Elizabeth and Uitenhage (Cape Colony) and renamed the parasite *Distoma capense*. In 1888, Dr Chute, of Kingwilliamstown, wrote an important article on the subject, connecting it with the Buffalo River. Dr Darley Hartley, when practising in Kingwilliamstown and Cathcart, also had many cases of the disease, traced to bathing in this same river. It is well known that it occurs in many other places in the Colony. Dr Saunders, of Grahamstown, mentions Keiskama, Alice, Konap, Fort Beaufort, as being villages from which he has received patients who were suffering from the complaint. Several natives suffering from the parasite told me they lived near and bathed in the St John's River in Pondoland. In Pietermaritzburg (Natal), European youths have been known to be extensively infected for many years. Dr Brock (1893, *Journ. Pathol. and Bacteriol.* vol. II. p. 52) has demonstrated the prevalence of the disease in the Rustenberg district (Transvaal), especially along the slopes of the Magaliesberg and Pilansberg mountains, and in the valley of the Eland, Hex, Magalies, and Crocodile Rivers. More recently, Dr Stock, Asst. M.O.H., Johannesburg, reported on an outbreak occurring among freshly imported English troops who had bathed in the Aapias river at Pretoria. The same author states that cases have been traced from the Orange River, Vaal River, Mnkanda (Newcastle, Natal), Mooi

River, Klip River, Umzindusi (Maritzburg), and streams in Middelburg (Cape Colony and Transvaal). In 1907, Capt. Gatt, R.A.M.C., contributed an article on the occurrence of the disease among European troops infected in Middelburg (Transvaal), and since then, Dr Arnold has reported that numbers of the civil inhabitants of Middelburg, who have bathed in either the Groot or Klein Oliphants River, have contracted the complaint. It is said that in the Klein Oliphants River there is one particular pool in which it is considered to be especially dangerous to bathe.

A statement has been made that people do not contract Bilharziosis in those rivers running west. This statement is borne out to some extent by references to Table I, which gives the result of microscopical examinations of samples of urines taken from natives of various tribes. It may be noticed that in no case were any Damaras or British Basutos found to harbour the parasites. Further, an educated British Basuto, employed in the hospital, tells me that he has never heard of people in his country passing blood with their urine.

The foregoing information collected from various sources should, with Table I, which gives results of urine examinations, and Table II, giving the number of times the parasites were found in livers at autopsies, make the geographical distribution fairly clear. These tables should also assist one in forming an estimate of the percentage of natives infected in each tribe, but a more accurate idea on this point can be obtained by referring to Table III, under the heading of Parasites.

Besides these:—

<i>Triodontophorus deminutus</i>	was found on 1 occasion,
<i>Physaloptera mordens</i>	„ „ 2 occasions
<i>Trichocephalus trichiurus</i>	„ „ 2 „

We see therefore that about 33·5% of natives have the *Bilharzia* parasite in the liver. The finding of the parasite in that position is often a matter of difficulty, consequently it must be admitted that any conclusions drawn from these figures will err on the small side.

The figures showing the numbers of other parasites found demonstrate the extent to which the South African native is infected. We may therefore consider the disease to be spread over the following areas. Commencing at the north, Nyassa, Mozambique and Quilimane natives are largely infected, that is to say, the people inhabiting the East Coast country between the southern border of German East Africa and

the Zambesi. South of the Zambesi, we find that natives recruited near Beira, on the banks of the Pungwe river, carry the parasite. In the area inland to this strip of coast country, we see in the Table II that a certain percentage of the British Central African natives, those from the eastern border of Lake Nyassa, the Angonis from the banks of the Zambesi, the Makalanga from Matabeleland, have the disease. On the coast line, south of latitude 22° S. are the Myambaams, Mtyopis, Shangaans and Lourenco-Marques boys; each of these tribes has a certain number of their people infected. That is to say, those people living between the Big Sabi river and Lourenco Marques. Inland of these coast countries, we have Swaziland and the Transvaal. Table I shows that some of the Swazis have the disease, and we know from reports concerning the European population that many parts of the Transvaal are grossly contaminated. The number of sick natives

TABLE I.

*Showing the results of microscopical examination for Bilharzia ova of a number of urines collected from various tribes of natives.*

Tribe	Number examined	Positive results	Percentage
Mozambique ... ..	190	48	25·8
Quilimane * ... ..	60	3	5
Lake Nyassa ... ..	22	8	36·3
British Central African ... ..	80	7	8·7
Shangaan ... ..	32	6	18·7
Mixed East Coast gang of Shangaan, Mtyopi, Myambaam, and Lourenco-Marques boys ... ..	225	26	11·5
Zulu ... ..	34	8	23·5
Pondo ... ..	10	2	20
Swasi ... ..	44	8	18·1
Mxosa ... ..	39	2	5·1
Transvaal Basuto † ... ..	59	1	1·6
British Basuto ... ..	68	—	0·0
Makalanga ... ..	45	10	22·2
Damara ... ..	50	—	0·0
Bechuana ... ..	12	2	16·6
Total	970	131	14·0

\* The results obtained from the examination of urines taken from these natives are interesting. The boys were not fresh recruits; they had all been working on the mines for 12 months. The ova in those urines giving positive results were few and far between. Post-mortem examination of fresh recruits show a very much higher per cent. infected. See Table III. This seems to suggest the advantage of removing patients from an infected area.

† The positive result obtained among the Transvaal Basuto was in urine from a boy who had been living in the Rustenburg district for some time.



TABLE II.

*Showing the number of times the Bilharzia worms were found at autopsies held on natives of various tribes. Also the number of times with which intestinal parasites were present.*

Tribe	No. examined	Bilharzia worms in liver	Ankylos- tomes	Round worms	Tape worms
Mozambique	215	102	139	17	1
Quilimane	129	60	81	32	1
Nyassa	52	16	27	10	2
British Central African	10	4	2	1	1
Beira	3	1	2	0	0
Angoni	14	2	3	1	0
Shangaan	65	23	32	27	2
Tonga	6	0	1	1	2
Myambaam	106	28	30	36	7
Mtyopi	40	9	15	8	8
Transvaal Basuto	12	1	1	0	1
Damara	19	0	2	1	0
Swazi	1	0	0	0	1
Pondo	6	0	0	1	3
Mxosa	5	0	0	2	1
Mixed Races	9	0	0	0	0
Total	692	246	335	137	30

from the Transvaal coming under my care is not so large as from other parts of South Africa. I have not therefore personally much evidence on the subject, but a medical missionary, Dr Liegme, in a paper he read before the Society for Advancement of Science in South Africa, states that haematuria is frequent in the Spelonken. Table I demonstrates the Zulu to be infected, and we know that the European population in parts of Natal likewise have the disease. I have had several cases among Amapondo and Amaxosa natives of Cape Colony, and as I have already stated the disease is prevalent in many places in the Eastern Province. Of the Western Province, I cannot speak definitely. British Basutoland has, so far, produced no patients for me. Damaraland is also free as far as I can judge by examining recruits from that country. Of the Orange River Colony, I am not in a position to speak decidedly, but I think one may anticipate that a certain percentage of the population on the north-eastern border will be infected.

*Source of Infection.*

That the disease is conveyed by some means from infected water there is no doubt, but except that the miracidium is killed by minute traces of hydrochloric acid not a single experimental fact has yet been brought to light indicating the actual source and mode of infection. The old idea in Cape Colony, still adhered to curiously by some, was that the organism in some form crept up the urethra while the persons were bathing. For this reason some of the inhabitants of Cape Colony used to tie their genital organs up in a handkerchief before going into a river to bathe. This custom may have induced some writers erroneously to try and prove that the prepuce cover worn by certain South African tribes was originally adopted as a protection against the parasite.

That infection occurs in some manner while bathing I think there can be little doubt. Dr Darley Hartley, when practising in East London and Cathcart, saw many cases of Bilharziosis. They always occurred in boys or young adults, and he tells me that in almost every case he obtained a history of them having bathed in the Buffalo River.

Dr Brock (1893, *Journ. Pathol. and Bacteriol.* vol. II. p. 52), relates an exactly similar experience when he lived in the Rustenburg district (Transvaal). Dr J. Allen (1888, *Practitioner*, vol. XI. p. 310) at one time Medical Officer for the Corporation of Maritzburg (Natal), writes as follows: "Nearly all the youths bathing in the Umzimdusi and Dorp Spruit are infected, while the girls, who do not bathe, remain free of the disease." In the two outbreaks among English troops in the Transvaal, the men infected had been bathing in spruits or rivers. The only case of a European girl becoming infected, that I can find notes of, is one reported by Dr C. P. Childe (III. 1899, *Brit. Med. Journ.* p. 644), and this girl, it was found on enquiry, had been in the habit of bathing in a freshwater pool on a farm in Natal. The facts seem to support the theory that infection occurs through the skin, for if the disease is contracted through drinking water, the girls would be infected as well as the boys, as, though the girls rarely bathe, they, in most cases drink the same water.

Moreover, when travelling among the natives on the East Coast, where both sexes drink the same water, and both bathe to about the same extent, I noticed that the women seemed, if anything, more commonly infected than the men; certainly the most severe types of the disease occurred among females. I was constantly requested to give medicines for women who were passing blood in their urine.

Dr Brock says that persons bathing in the streams skirting Rustenburg complain of severe itching when emerging from the water. Dr John Harley noticed that people in Cape Colony often suffered from nodular excrescences of the skin, which later became transformed into indolent ulcers. He considered these consequent upon the invasion of the parasites into the skin when bathing. Personally I have not come across any skin eruptions which in any way suggest the entrance of any kind of embryos or larvae, unless perhaps a native skin disease known as Dwappo.

I think the preceding statements make it far more probable that infection does not occur by the drinking water, but rather through the skin while a person is wading or washing in infected rivers or pools, the worm entering in the same manner as the larvae of the *Ankylostoma duodenale*.

It has been urged against the probability of infection occurring through the skin while bathing, that monkeys contract the disease, and these animals certainly do not bathe, while they constantly drink water from streams or pools when they cannot get dew, and in doing so, probably immerse their feet at the edge of the water. It seems to me most probable that the disease is contracted either by bathing or wading in infected water or mud.

I may add here that Europeans have noted that certain pools in the rivers are more fruitful in producing cases of the disease than others. The natives on the East Coast state that some cases are produced by bathing in certain pools, but they also state that it is caused by drinking M'Jumbula spirit (a filthy spirit made by the distillation of a preparation of manioca root)!

Cobbold originally suggested that there must be an intermediate host in the life history of the parasite, but to the discussion of this subject I am not in a position to add any fresh information.

#### *Incubation Period.*

Dr Stock in his article on *Bilharzia*, published in the *Lancet* of September 29th, 1906, quotes the cases under Dr Abercrombie's charge which occurred among newly arrived drafts of young soldiers who bathed in supposedly infected pool near Pretoria. Of these, the shortest time in which symptoms developed after bathing was one month, the longest two months. He accordingly concluded that the incubation period lasted about six weeks.

In connection with this it must be remembered that people can probably carry the parasites for some considerable time without any signs of the disease being apparent, provided they lead a quiet life, but the symptoms may at any time appear suddenly in consequence of some severe exertion. That the period of incubation according to Stock is shorter than that observed by others may be due to the fact that the troops in question were cavalry, subject to rough riding; a form of exercise most likely to bring on the symptoms at an early date.

### *Symptoms.*

Before discussing this portion of the subject, I must state that a number of my patients spoke a language understood by no Europeans that I know, and by very few natives. Consequently, to obtain any account as to how the patient really felt was difficult and to obtain anything approaching an accurate history of the cases was impossible. But the symptoms I noted do not appear to vary much from those reported, as occurring among the inhabitants of other countries. It is therefore only necessary to discuss them briefly.

They appear to vary in severity in different localities. Consider for example the case of European and native youths in parts of the Transvaal, Natal and Cape Colony, where, though numbers are infected, the majority only have a certain amount of haematuria; they may at times show evidences of debility, but they are not often really seriously incapacitated. On the other hand, among the natives on the East Coast and more tropical parts, there is, as stated before, probably no disease which causes the inhabitants so much worry as Bilharziosis.

This may probably be due to there being more than one species of parasite; the population of the southern parts are only infected with the variety of disease which attacks the urinary system; that of northern and tropical parts harbouring both kinds. At the same time, it is also possible that the infection in the northern districts may be more extensive than in the southern.

The individual symptoms may be divided into those affecting (1) the urinary system; (2) the intestines.

### *Urinary System.*

Haematuria is the most prominent and important symptom of them all.

It occasionally appears suddenly; the result of an accident. I had

a patient, a young Pondo, who told me he first noticed that he was passing blood in his urine after a fall from a horse.

In some cases, though the urine appears quite clear, if it is treated in a centrifuge, and then examined under a microscope, red blood corpuscles and a few ova will be found. At other times, the whole urine passed is faintly blood-stained, or the first water evacuated may be quite clear, a few drops of pure blood only appearing at the end of micturition. With some cases, small tube-shaped blood clots are passed in the urine stream. In the most severe cases, almost pure blood is emitted and occasionally this may coagulate in the bladder so firmly as to cause complete suppression of urine. I have only seen the last condition once; it was a case of a European who came from the Rustenburg district.

With severe haematuria cystitis often develops, thus necessitating frequent micturition, which, with the anaemia and the mental worry, has a very debilitating effect on the system. I must, however, admit that I have never had a native or European die of uncomplicated *Bilharzia* disease of the bladder, though I have seen some excessively severe haemorrhages as a result of it.

The haematuria is frequently brought on by violent exertion, especially riding.

On several occasions, I have had patients admitted to hospital who rolled on the floor in intense pain, which they referred to the pubic region; they had apparently no other symptom, though one native had incontinence of urine. With a catheter a small amount of purulent urine, containing *Bilharzia* ova, was drawn off. When I first saw these patients I thought they had received an irritating poison, and that possibly some native doctor had been administering a mixture somewhat more searching in its effects even than those they usually employ. However, hot fomentations, hypodermic injections of morphia and urotropine administered by the mouth, relieved the symptoms, and after a few days the patients recovered sufficiently to be transferred to the convalescent room. They remained fairly well there for a week or two. After this time, they were readmitted to hospital with indefinite symptoms and often died suddenly within a few days. At other times, these patients lay wrapped in their blankets in a drowsy condition for days and weeks. Beyond the fact that their urine contained albumen, which is so common an occurrence among native patients, that one has to disregard its presence, there were no symptoms indicating the cause of the condition. The temperature on admission usually being above

normal, but within a few hours falling to subnormal and remaining so until death.

A post-mortem examination usually showed that all the organs except those of the urinary system were healthy. But the kidneys were in a state of profound hydronephrosis, the organs being mere shells.

#### *Urinary Fistula.*

In accounts of the disease by Egyptian observers fistula is noted as a common complication.

The number of natives I see suffering with fistula is small. I have never seen one which I suspected was due to Bilharziosis. But most of my patients are adult males in the prime of life; the condition may be more frequent among the old men, unfit and unlikely to seek for work away from their own country, though when I have been in the kraals I have not noticed an abnormal number of perineal complaints.

Of the many complications which must occur when the parasites attack the genito-urinary organs of the female I have had no experience.

#### *Intestinal Bilharziosis.*

The symptoms of this condition in my experience are exceedingly indefinite; they are most liable to be mistaken for those of chronic diarrhoea or dysentery.

Those cases I have diagnosed during life have been identified more by the exclusion of other diseases than anything else. Because though I make microscopical examinations of the faeces, the number of ova found in the specimens often did not convey any correct impression as to the extent of the disease. It seems that the ova really causing the mischief are those working in the tissues. One may even, in severe cases of Bilharzial infection of the intestine, experience a little difficulty during life in demonstrating the ova, though after death a scraping from the mucous membrane of the rectum shows the tissues to be infiltrated with them.

This peculiarity of the disease accounts for it not being more often recognised when examinations of faeces are made say for the ova of *Ankylostomum duodenale*. When I see a patient suffering from chronic diarrhoea passing five to six motions daily without much slime or blood, and not responding to ordinary treatment, but wasting and becoming anaemic to a degree quite out of proportion to the extent of

the diarrhoea, I suspect Bilharziosis. I do not wish to infer that my patients never pass blood or slime in their motions but, as a general rule, they have not done so; their motions recall those of a patient with acute tuberculosis, complicated with diarrhoea.

Straining and tenesmus have not been marked features of the disease, though one patient was in agony for several days before death unless under the influence of morphia. I particularly lay stress on the absence of these symptoms, because I note in other countries they are reported as being marked features of the disease. It may be interesting to note here that, though I have experienced some most virulent epidemics of dysentery among natives, attended by a very heavy mortality, tenesmus, which is most frequently a very trying feature of the disease when Europeans are attacked, was comparatively uncommon among my coloured patients.

#### *Temperature.*

The temperature in intestinal Bilharziosis cases under my notice has been, as a rule, normal or subnormal, varied at intervals of seven or eight days by a two or three days irregularity. This disturbance may have been of malarial origin; at the same time I suggest it may have been due to a crop of embryos or miracidia having burst from the ova into the tissues.

#### *Duration of the Disease.*

In Cape Colony and more southern parts of South Africa one generally expects the symptoms to abate and finally disappear when the patient reaches to about twenty years of age. But that the disease may persist for a long time without any re-infection occurring is proved by Maj. Freeman (III. 1907, *Journ. Roy. Army Corps*, vol. VIII. p. 278) who quotes the case of a soldier who was infected in Pretoria and afterwards returned to England. Three years after his return, living ova could still be found in his urine. I am inclined to think it doubtful whether a person, once he has contracted the disease, and the walls of his bladder and the surrounding tissues have become impregnated thoroughly with calcified ova, will ever be completely free of all evidences of the complaint. I think that years after all active mischief has ceased, scraping of the bladder tissue would still show calcified ova, or their débris. That is to say, the ova can be demonstrated long after the actual trematode producing them has been destroyed and absorbed.

*The Parasites.*

I do not intend to enter into the anatomical description of the actual parasites or to give an account of the dimensions or other minute details of their ova. That is a matter to my mind requiring the knowledge of an expert helminthologist.

The natives of South Africa are infected then with the two forms of the disease, viz. the classic form and Manson's Bilharziosis.

I have found both terminal and lateral spined ova at autopsies and when examining the excreta from patients in hospital, and I believe the parasites which deposit these two distinct forms of ova normally infest different organs, though the terminal spined ova occasionally trespass on the preserves of the lateral spined one.

I have been able to find the worms most readily in the portal veins of the liver (see Table II), but the parasites in that situation are, I understand from Dr Leiper (London School of Tropical Medicine), to whom specimens were submitted, of the male sex and immature. To find the mature worms it is necessary to examine the ramifications of the venous system of the bladder and rectum. I have never found worms or ova in the arterial system.

Some of the ova probably remain in a calcified state long after the worms have disappeared from the system. That there may be two separate trematodes is, I think, supported by the fact that the terminal spined ova is the only variety ever found by me in the urine or mucous membrane of the bladder and ureters<sup>1</sup>.

In the faeces and mucous membrane of the large intestine lateral spined ova are generally found, with sometimes, however, a few terminal spined ones amongst them, and these latter are frequently in a calcified state, suggesting that they have worked their way from the bladder tissues rather than that they have been deposited by the blood stream. On four occasions at autopsy (examinations on two Angonis, one Shangaan, and one Quilimane native) I found lateral spined ova in the mucous membrane of the large intestine, but although I carefully scraped and examined every portion of the mucous membrane of the bladders, no ova of any kind could be found in these organs.

My observations of the occurrence of the lateral spined ova are

<sup>1</sup> In a discussion reported in the *Lancet* of April 20th, 1889, Dr Moon stated that "he had found both terminal and lateral spined ova in both bladder and rectum." This statement does not hold good in South Africa.



confined to what is known as the low veldt in South Africa. I have not found lateral spined ova in natives coming from other than tropical or semi-tropical parts. There is too the marked difference in the severity of the general symptoms of the disease in the two localities.

To demonstrate approximately the number of times the terminal spined ova were produced as compared with the lateral spined ones, and also to ascertain in what proportion of cases both kinds of ova could be found in the same person, I made a careful microscopical examination of scrapings taken from the mucous membrane of the bladders and large intestines obtained at 90 consecutive autopsies. These examinations were conducted with special care, and I think the results given in the following Table III may be relied on absolutely.

TABLE III.

*Showing results of microscopical examination of scrapings taken from the mucous membrane of bladders and large intestines at 90 consecutive autopsies; also showing the number of times the Bilharzia worm was found in the vessels of the liver.*

Tribe	Total no. of examinations	Bladder			Large intestine				Liver	
		Terminal spined ova	Lateral spined ova	Not microscopically examined*	Lateral spined only	Terminal spined only	Mixed lateral and terminal spined ova	Not microscopically examined	Worms present	Not found
Myambaam	11	10	0	0	2	1	2	0	3	8
Mtyopi	5	5	0	0	3	0	0	0	1	4
Shangaan	8	7	0	0	2	1	0	1	2	6
Nyassa	3	3	0	0	0	0	0	0	1	2
Mozambique	30	24	0	2	3	5	3	4	10	20
Quillimane	15	12	0	0	4	0	2	2	4	11
Angoni	15	11	0	2	4	1	2	3	3	12
Transvaal Basuto	3	2	0	0	0	0	0	0	0	3
Total	90	74	0	4	18	8	9	10	24	66

\* A few separate organs escaped microscopical examination for unavoidable reasons; the numbers so escaping have been noted in a special column.

In four of the above cases, namely, two Angonis, one Shangaan, and one Quillimane native, lateral spined ova were found in the mucous membrane of intestine but no ova could be found in the bladder. In only nine cases was the *Bilharzia ovum* absent in all organs. One Mozambique native gave negative results in the bladder and intestine but a single *Bilharzia* worm was found in the liver. The numbers of terminal spined ova found in mucous membrane of the large intestine were usually very few.

To Table III, I may add that I found the terminal spined ova in the following organs:

- |              |                                |
|--------------|--------------------------------|
| (1) Bladder. | (5) Veins of foregoing Organs. |
| (2) Ureters. | (6) Prostate Gland.            |
| (3) Kidneys. | (7) Mesenteric Gland.          |
| (4) Rectum.  | (8) Lungs.                     |

I have not examined the heart or pancreas for ova, and on only two or three occasions, the spleen substance. I examined the urethra on several occasions, always with negative results.

The lateral spined ova I found principally in the rectum and occasionally a few in the liver. It is interesting to note that I have not found the terminal spined ova in this last organ, though I expected to do so.

The above facts seem to point to a wide area of location of the terminal spined ovum, but a much more restricted field for lateral spined ova.

#### *The Ova.*

The following is a rough description of the two forms of ova, as I have seen them, which may be of interest, though it does not enter into minute details.

#### *Terminal Spined Ova.*

These may be observed in three forms:—

(a) The light coloured animated ovum with the clear well marked contents.

(b) The ovum with homogeneous black contents, which are apparently calcified, evidently dead and incapable of doing any more damage to the tissues than coarse pigment granules would do.

(c) Shells of ova from which the miracidia or embryos have escaped.

Those ova found outside the urinary system or intestines were, to the best of my recollection, all of them in the calcified condition described under heading (b).

#### *Lateral Spined Ova.*

These may also be seen in several forms:—

(a) The light coloured animated ovum with the prominent lateral spine.

(b) The light coloured animated ovum, with a spine looking like a thin thorn projecting from the surface.

(c) The ovum with a very thick spine, producing the appearance almost of a division of the lower end of the egg. This represents, I believe, the calcified state in the terminal spined ovum.

(d) Shells of the lateral spined ova from which the embryos have escaped.

As regards the first two forms of the lateral spined form, it must be noted that at times, on first looking at them, it appeared as though they had no spines at all. But immediately on pressure being brought to bear on them through the cover-slip, a spine shoots out. I think this is in most cases an optical delusion, and that what really happens is that the ova were lying with the spines on their inferior surface, and that pressure on the cover-slip causes the eggs to turn over, so bringing the spine suddenly into view.

I may mention that I frequently, when examining scrapings from the mucous membrane of the bladders and intestines, found empty ova shells, from which the miracidia or embryos had escaped. In Cobbold's *Entozoa*, it stated that Griesinger found a number of empty eggs in the left ventricle of the heart. Other authorities have doubtless observed the same fact. I have also, when examining perfectly fresh specimens, especially those taken from the mucous membrane of the rectum, observed that the slightest pressure has caused the ova to burst and the field of the microscope has been suddenly populated by numbers of rapidly moving miracidia. These statements suggest to my mind that the ova do occasionally dehisce while still within the human system, and explain occasional evidences of recrudescence of the disease.

### *Pathological Anatomy.*

The changes caused by this parasite are principally to be seen in the urinary and alimentary systems.

#### (1) *Urinary System.*

*Calculus.* I am dealing with this subject separately, as I consider my observations are of especial interest. I notice that in Egypt the frequency with which natives suffer from stone in the bladder is ascribed to Bilharziosis. In South Africa, of the 66 autopsies I have made on natives infected with the disease, in which the bladder has been particularly examined, I have not once found a stone. Further, I

have never had a patient presenting the clinical symptoms of calculus of the bladder. In view of Egyptian records this experience appears extraordinary.

*Bladder.* This organ is the one most commonly affected. As stated before, I have only made four autopsies on natives found to harbour the parasite, in which the bladder escaped infection. The effect of the ova on the coats of the organ varies greatly, according to the numbers present, and to the length of time they have had to produce the changes.

In some the only feature noticeable on first opening the bladder is a small slightly inflamed spot on its mucous membrane; in others, one is at once struck by the presence of several acutely congested papilliform bodies projecting into its interior, which would undoubtedly bleed when squeezed by the contraction of the bladder at the end of micturition. In more advanced cases still, the area of acute congestion is extensive, affecting nearly the whole of the internal surface, in the centre of which there may be a patch of black gangrenous mucous membrane, easily detachable. Such a condition might result in a very large haemorrhage. It would appear that the varying degrees of inflammation described above represent the sub-acute and acute stage of the disease, and that at a later date, when the parasite dies out, or the ova for some reason lose their activity, other changes occur, which can be looked upon as representing a more or less chronic condition.

I make this statement, because, in other bladders I have found on careful examination faintly coloured brownish spots on the mucous membrane, not gritty to the touch, or raised above the surface, in which a few terminal spined calcified ova can be found on microscopical examination. This condition is, I believe, the chronic stage of the sub-acute condition described first, in which there was only very slight congestion.

Following this stage a little further, one finds bladders in which there are four to five greyish-brown patches, of about 15 mm. in diameter, slightly raised above the surface of the mucous membrane; these when scraped with a scalpel can be heard and be felt to be gritty. Later still the spots are much more elevated, and have blackened tops, which project into the bladder, and resemble warts which have been burned with caustic. In other bladders, nearly the whole extent of the internal surface is affected. Such bladders when opened and spread out, resemble a sheet of very fine sandpaper, and on the surface of some of them I have seen small pin-head vesicles, which are hard, feeling like

small nodules of cartilage. They have an opalescent appearance, glistening in bright sunlight. If one of these vesicles be removed and placed under a cover-slip it takes a certain amount of pressure to burst it. Microscopically, it consists of a capsule containing a little clear or cloudy fluid, in which is suspended a nest of black terminal spined ova, or the debris of ova. I think it may be stated that the various coats of the bladder become thickened to a greater or less degree in every case of the disease (due to the interstitial growth of fibrous tissue. See Bowley, *Lancet*, April, 1889). In some, the thickening is very slightly marked, but in others they may be half an inch thick, like the walls of a uterus. Such a condition greatly reduces the capacity of the organ, and at the same time diminishes its power of expansion.

*Ureters.* These are the organs which, next to the bladder, are most commonly affected. The portions of the ureters which pass through the bladder wall, and the first two inches of their lower ends, are the parts usually implicated. Occasionally patches of infection of about an inch in extent, may be found midway between the bladder and the kidneys, sometimes even nearer than this to the latter organs. The conditions of the mucous membrane of these tubes vary markedly. In some the affected parts have a soft pale cream-coloured velvety appearance. In others there are dark brown gritty patches. When such occur near the bladder, the lumen of the tube is frequently distended, and almost blocked with masses of brown sandy material looking somewhat like coffee grounds. On one occasion I removed about half a tea-spoonful of this substance from each ureter, which microscopically I found to consist of black terminal spined ova and their debris.

Once I found the mucous membrane sprinkled with dark specks like black powder grains. These were exceedingly hard. I broke a cover-slip in crushing one of them. I believe they consisted of calcified debris of ova, and that they are a secondary condition to the little vesicles already described as appearing in the bladder, as I found similar vesicles in the ureters in close proximity to the black specks. On one occasion the lumen of the left ureter was completely obliterated in four places, and the kidney on the corresponding side was cystic and atrophied. With adherent tissues it only weighed 37 grammes.

The walls of the ureters become hypertrophied in the same manner as do those of the kidneys.

*Kidneys.* Calculus. On only one occasion have I found a stone in the kidney. It was about the size of a millet seed and lay in the calyx of the left organ.

Though I have constantly traced the *Bilharzia* ova from the bladder up the ureters to hilum of the kidney, it would seem that it is comparatively rare for the kidneys themselves to be affected by the disease in its acute stage, the ova not being found in the substance of these organs in large numbers. But the kidney is sometimes markedly affected secondarily to the pathological changes in the bladder and ureters. There are three main causes inducing kidney changes: (1) as the coats of the bladder become thickened they tend to cause a partial occlusion of the lumen of the ureters passing between them; (2) the deposit of coffee-ground material already described, at the lower end of the ureters, reduces their lumen; (3) the thickening of the coats of the ureters has the same effect.

These three conditions retard the urine from entering the bladder, and set up a back pressure, which eventually causes hydronephrosis and later pyonephrosis, in some cases to such a degree that after death the kidneys are found to be mere shells. The following table shows the number of times, in 420 autopsies, in which there were cysts in the kidneys or the calices were dilated.

TABLE IV.

Tribe	Number examined	Cystic kidney	Per cent.
Nyassa	86	0	0·0
Mozambique	160	15	9·4
Quillimane	56	6	10·7
Beira	2	0	0·0
Myambaam	64	7	10·9
Mtyopi	24	0	0·0
Shangaan	38	1	2·6
Tonga	2	0	0·0
Angoni	15	1	6·7
Mashona	1	0	0·0
Basuto	7	1	14·2
Mxosa and Pondo	5	0	0·0
Damara	10	1	10·0
Total	420	32	or 7·6

I have seen it stated that the kidneys of persons affected with *Bilharziosis* weigh more than the normal. This, one would naturally expect to be the case, but my limited figures do not bear the statement out, as, out of five cases which I proved microscopically did not contain the ova in the bladder, the average weight of the two kidneys was 284·4 grammes, whereas the average weight per pair of fifty pairs of kidneys

taken from bodies proved microscopically to have the ova in the bladder was only 270·75 grammes. The figures are too few to place much reliance on them, but they strike one as peculiar. Of the other parts connected with the genito-urinary system, I shall refer to :

*Prostate gland.* I have frequently found a few nests of terminal spined ova in this gland. I have not seen any marked naked-eye lesion in it, and my attention has not been called to it clinically in any way.

*Urethra.* Though I have examined the lining membrane of this tube microscopically on several occasions, I have never found it to be affected. But I note that in other countries the urethra is sometimes attacked.

*Foreskin.* On several occasions I noticed a hypertrophied condition of the foreskin, which I suspected to be caused by Bilharzial infection but I have never confirmed the diagnosis.

*Alimentary canal.* The large intestine is the portion of the alimentary canal most frequently affected. In some cases the mucous membrane of the lower bowel is acutely congested, without any evidence of ulceration. At other times the whole surface seems honey-combed with clear punched-out ulcers of about the size of a split pea. In others again, there is a granulating condition, reminding one of the surface of the skin following a burn, in that state which, as a student, I was taught should be rubbed with bluestone before any attempt should be made to skin graft. At a later stage to this a papillomatous condition is found, reminding one of haemorrhoids.

Besides these acute conditions a stage is often found, which I believe corresponds to the chronic sandy stage in the urinary system—black leaden-coloured patches of mucous membrane without ulceration, a deep scraping from which shows black lateral spined ova. In some, this pigmented state may be so marked that the whole surface of the mucous membrane looks like a strip of black velvet. This peculiar colouration has always been associated with lateral spined ova. As in the bladder, so in the intestines the various coats are greatly hypertrophied.

I have not looked for the disease in other parts of the alimentary tract, but Dr May, the Government Bacteriologist, examined a cyst from the stomach of an imported Chinaman, which contained terminal spined ova.

*Liver.*

The numbers of South African natives who contract a cirrhotic condition of the liver is, I think, large. In the following table, No. V, we see that out of 739 post-mortems, the liver was found to be cirrhotic on 106 occasions, or 14·3%.

TABLE V.

*Table showing the number of times in which at 739 autopsies the liver was found to be cirrhotic.*

Tribe	No. of examinations	Cirrhotic liver	Percentage
Nyassa	53	5	9·4
Mozambique	221	27	12·2
Quilimane	182	29	21·9
Myambaam	114	21	18·4
Mtyopi	44	7	15·9
Shangaan	74	10	13·5
Tonga	6	1	16·6
British Central Africa	15	3	20·0
Matabele	1	0	0·0
Makalanga	4	1	25·0
Transvaal Basuto	11	1	9·1
Zulu	1	0	0·0
Mangwato	2	0	0·0
Rhodesian	2	0	0·0
Beira	2	1	50·0
Angoni	15	0	0·0
Mashona	1	0	0·0
British Basuto	2	0	0·0
Pondo	8	0	0·0
Mxosa	4	0	0·0
Fingo	1	0	0·0
Damara	26	0	0·0
Total	739	106	or 14·3

Whether this cirrhosis is entirely due to *Bilharzia* is, I think, doubtful, as, though when we analyse the Table, we see that the tribes with the highest percentages affected with cirrhotic livers come from those areas where the lateral spined ovum is most prevalent, yet it must not be forgotten that these are also the very districts where malarial fever exists in its worst forms, and where, above all, alcohol is consumed by the natives in enormous quantities. In the Portuguese Territories natives can obtain as much imported spirit as they can pay for. Furthermore, numbers of them are adepts at the distillation of



spirits. Provided with a couple of kaffir pots and an old gun barrel, the native soon starts a very efficient distillery. The country abounds with vegetable products from which he can make spirits, the two commonest of which are, I think, the Cashew apple and the Manioca root. In consequence of the ease with which a native can obtain alcohol, he consumes, as stated, enormous quantities, and I think it may truly be said that, in certain seasons of the year, he is never properly sober.

If we consider the other liver complaints from which the native suffers, viz. waxy disease or fatty degeneration, we find them to be above the normal, as shown in the following Table:

TABLE VI

*Table showing number of times the liver was found to be either waxy or fatty at 400 autopsies.*

Tribes	Number examined	Waxy	Fatty
Shangaan	38	4	3
Myambaam	64	4	3
Mtyopi	24	3	0
Nyassa	36	1	1
Mozambique	180	2	6
Quilimane	56	1	3
Beira	2	1	0
Total	400	16	16

I do not think that it has been suggested that Bilharziosis is responsible for either of these conditions.

The trematode itself is not found most frequently in the cirrhotic livers, as, out of a hundred consecutive autopsies, fourteen showed a cirrhotic condition of the liver, and in these the worms could only be found twice, whereas they were found twenty-four times in the remaining livers, which were either normal or merely congested. This feature may be due to the fact that the cirrhotic liver has often very little blood in it, consequently it is difficult to express the worms. And it is also possible that the worms only stay in the liver when in an immature state, therefore not laying eggs; that at maturity they seek other spheres, and the eggs then laid are carried back to the liver and set up the cirrhotic condition.

I am not in a position to speak too definitely on this matter, but the number of ova found in the liver tissues does not appear to be sufficient to warrant the conclusion that they are responsible for the cirrhosis.

It is generally accepted that the ova, and not the worms, cause the pathological changes.

#### *Mesenteric Glands.*

These glands in a patient with intestinal Bilharziosis are slightly enlarged: they are of a very dark colour and of hard consistence. I have frequently found terminal spined ova when examining scrapings taken from them.

#### *Lungs.*

Of the few lungs I have examined I have only found the ova on one occasion (terminal spined ova) in a boy who died from acute pulmonary tuberculosis. The tuberculosis was evidently secondary to the Bilharzial infection. It is reasonable to suppose that a Bilharzial infection of the lungs would render the patient more susceptible to infection with *Bacillus tuberculosis*. It is interesting to note that Cobbold suggests the peculiar susceptibility of the South African native to pneumonia may be the result of Bilharzia.

#### *Treatment.*

I have only heard of three native remedies, but doubtless many others exist: (1) A decoction made from the roots of a *Cassia*, growing in Equatorial East Africa, which Dr O'Sullivan Beare obtained from the natives of those parts. This plant has since been named *Cassia beareana*; an extract made from the roots has been used with marked success in the treatment of Blackwater fever (see O'Sullivan, *Lancet* of Feb. 1st, 1902, and Bostock, *Transvaal Med. Journ.* of July, 1907). (2) While among the kraals in the Inhambane District, I was informed by natives that they used a medicine, consisting of a decoction of the leaves of a tree called *Umrangala Umgubo* (spelt phonetically). The use of this plant for bladder troubles would appear very widespread, as a Mozambique boy told me that a decoction of the roots of the same plant was used in his country by persons passing blood in their urine. Among the Mozambiques, he informed me, the plant is known by the name of *Reba Reba*: I regret to say that I have not been able to get this plant identified. (3) A demulcent drink made from the beard of mealie cobs has a great reputation for the treatment of the disease among some natives.

While suffering from an attack of the disease natives in Portuguese Territory abstain from eating Cashew apple (*Anacardium occidentale*) and from drinking wine or spirit manufactured from it. As stated previously, some natives attribute the disease itself to the drinking of manioc spirit.

Küchenmeister's Manual suggests the use of onions and garlic in the treatment of the disease. Griesinger prescribed a mixture of calomel and turpentine.

Methylene blue has a reputation in some parts. Liquid extract of male-fern in 10 m. doses, twice daily, has its advocates. Santonin, quinine, cubeb, sandalwood oil, benzoic acid, have all been recommended.

Personally I have obtained the best results in the treatment of Bilharziosis affecting the bladder by the use of urotropine, 5 gr. doses night and morning, in half a pint of water, with rest in bed. Should the haemorrhage become severe I use adrenalin. With this treatment I have always been able to quiet the symptoms, but I am inclined to think that I have been singularly fortunate in the class of patient with which I have had to deal.

As regards the treatment of the intestinal disease, I have to admit that I know of no drug which has the slightest influence on it. I have used infusion of monsonia the most frequently, after that magnesium sulphate, opium, astringent remedies and other drugs. I may almost say that I have worked steadily and conscientiously through the British *Pharmacopoeia* without any satisfactory result.

### *Prognosis.*

This varies according to the system of the body which is infected. If it is purely an uncomplicated case of terminal spined ova affecting the bladder of a male, the consequences are not as a rule very serious in South Africa. If, on the other hand, the disease has obtained a firm hold of the mucous membrane of the large intestine, I consider the prognosis of the case a grave one.

## NOTE ON SPIROCHAETES IN CASTRATION TUMOURS OF PIGS.

By J. BURTON CLELAND,

*Government Pathologist, Perth, Western Australia.*

*(From the Pathological Laboratory, Department of Public Health.)*

IN pigs in Western Australia it is of frequent occurrence to find at the seat of castration large oval fibrous tumours from the size of an hen's egg to that of a tennis ball. These have a thick fibrous wall with a cavity in the centre, which is frequently small in relation to the size of the mass. The walls of this cavity are brownish-yellow and degenerated and the contents usually sero-pus of a similar colour, though at times a large quantity of ordinary whitish purulent matter is found. In films made from this brownish-yellow pus and stained with weak carbol-fuchsin or Leishman's stain, varying numbers of spirochaetes mixed with minute cocci and bacilli and larger, occasionally spore-bearing, organisms will be found. These spirochaetes vary in length from  $6\mu$  or less to  $12\mu$ ; their thickness varies from the most delicate tenuity to that of a tubercle bacillus; the spirals may be three or four and perfectly regular or, on the other hand, quite irregular, and acute bendings even at a right angle may be seen. Further, some of the large apparently bacillary organisms may show slight undulations suggesting that they are large forms of this spirochaete. Sections of the tumour show a fibrous stroma, becoming more cellular towards the centre where it passes into necrosed tissue swarming with organisms, amongst which, sometimes in masses, spirochaetes may be found. Where the still living cells abut on the necrosed area, a varying number of eosinophile cells are revealed by Leishman's stain.

The occurrence of this spirochaete is especially interesting when viewed in connection with the presence in man under certain conditions of *Spirochaeta refringens*. Whether the spirochaete found in the

tumours has a definite etiological relation to their formation or is only an accidental denizen of their cavity, introduced at the time of castration with the organism causing the swelling, it is impossible to say.

Recently Sydney Dodd, in the *Journal of Comparative Pathology* vol. XIX. 1906 (vide note, *Journal of Tropical Vet. Science*, vol. II. no. 2), has described an ulcerative skin disease of pigs, in which the ulcers contained spirochaetes apparently somewhat similar to these.

## NOTES ON THE DRUG TREATMENT OF CANINE PIROPLASMOSIS.

BY GEORGE H. F. NUTTALL, M.A., M.D., PH.D., SC.D., F.R.S.  
AND G. S. GRAHAM-SMITH, M.A., M.D.

THE observations relating to the treatment of canine piroplasmosis by means of drugs are usually very briefly recorded so that the precise conditions under which they were conducted cannot be ascertained.

In South Africa Hutcheon (1893, p. 477, and 1899, p. 400) recommended the use of repeated doses of ammonium chloride and belladonna, a form of treatment tried by Borthwick at Port Elizabeth with "excellent results." Subsequently Hutcheon obtained encouraging results from the use of quinine, benzoate of soda and carbolic acid. Robertson (1901, p. 332) tried calomel, quinine, ammonium chloride, extract of belladonna, carbolic acid and benzoate of soda without success. In fact he thought that carbolic acid hastened death. He obtained the best results from a "calomel pill to start with, then a calomel and quinine pill four times a day." Without stating the dose he says that very large amounts of calomel are needed. Hutcheon, on the other hand, did not approve of the calomel treatment.

In Europe Piana and Galli-Valerio (1895) attributed the recovery in one case to the use of quinine. Almy (10. x. 1901, p. 379) treated dogs with quinine hydrobromate, but found it to be as ineffective as quinine has been shown to be in the treatment of Tristeza (piroplasmosis of cattle).

Though various other remedies have been suggested and tried, none have yet been discovered which produce any marked effect on the course of the disease. In a few cases successful results have been claimed, but importance cannot be attached to these isolated instances since dogs occasionally recover without treatment.

We have made a few carefully conducted experiments with selected dogs. In each case two dogs of about the same age and size were inoculated at the same time with similar doses from the same specimen of defibrinated heart blood obtained from a dog dying from the South

African strain of *P. canis*. One dog was kept as a control and the other treated. One experiment was made with quinine bihydrochloride, two with tartar emetic, a drug which has been used with some success in trypanosome infections, two with sodium-methyl-arsenate (combined with calomel), and two with methylene blue, drugs which are reputed to be of value in the treatment of this disease. Two experiments were also made with Beta-naphthylamine. In no case however was the course or duration of the disease influenced in any way by the treatment.

The records of our own experiments and of those of Gonder with atoxyl are given below.

*Quinine bihydrochloride.*

1. Dog I (puppy).

Day 1. Inoculated with 3 c.c. of defibrinated heart blood.

5. 2—3 infected corpuscles observed per field.

Treatment begun: 2.5 grains of quinine bihydrochloride dissolved in 1 c.c. of normal salt solution injected subcutaneously twice a day.

A 5 grain calomel pill also given daily.

6. Treated as before, but the second dose of quinine raised to 5 grains.

7. Found dead.

*Control dog* (puppy) found dead on 6th day.

*Result.* Treated dog died on 7th day, control dog on 6th day.

*Tartar emetic.*

2. Dog II (puppy).

Day 1. Inoculated with 3 c.c. of defibrinated heart blood.

3. Treatment begun before the appearance of the parasites. It consisted of daily subcutaneous injections of 20 minims (1.18 c.c.) of 0.25% tartar emetic.

8. A few parasites observed in the blood.

10. Many parasites in the blood. Haemoglobinuria.

11. Found dead.

*Control dog* (puppy) killed when dying on the 9th day.

*Result.* Treated dog died on the 11th day, and control dog on the 9th day.

3. Dog III (puppy).

Day 1. Inoculated with 4 c.c. of defibrinated heart blood.

6. Treatment begun: subcutaneous injections of 20 minims (1.18 c.c.) of a 0.5% solution of tartar emetic given daily.

7. A few parasites observed in the blood.

9. Many parasites in the blood. Haemoglobinuria.

10. Found dead.

*Control dog* (puppy) died on the 10th day showing many parasites in its blood.

*Result.* Treated and control dog both died on the 10th day.

*Sodium-methyl-arsenate.*

## 4. Dog IV (puppy).

Day 1. Inoculated with 3 c.c. of defibrinated heart blood.

5. 2—3 infected corpuscles observed per field. Treatment begun : 2.5 grains of sodium-methyl-arsenate administered twice daily in a bread pill, together with a 5 grain calomel pill once daily.

6. The afternoon dose of arsenate raised to 5 grains.

7. 5 grains of arsenate given in the morning.

Died at mid-day ; many parasites in the blood.

*Control dog* (puppy) found dead on the 6th day.

*Result.* Treated dog died on the 7th day and the control on the 6th day.

## 5. Dog V (puppy).

Day 1. Inoculated with 3 c.c. of defibrinated heart blood.

8. A few parasites observed in the blood. Treatment begun : 5 grains of arsenate and a 5 grain calomel pill given once daily.

9. Many parasites in the blood. Haemoglobinuria.

10. Found dead.

*Control dog* (puppy) showed many parasites on the 3rd day and died on the 6th day.

*Result.* Treated dog died on the 10th day, control dog on the 6th day.

*Methylene blue.*

## 6. Dog VI (puppy).

Day 1. Inoculated with 3 c.c. of defibrinated heart blood.

4. A few parasites observed in the blood. Treatment begun : 1 grain of methylene blue administered daily in the form of a pill.

7. Many parasites in the blood. Dog died.

*Control dog* (puppy) died on the 7th day with many parasites in its blood.

*Result.* Treated and control dogs both died on the 7th day.

## 7. Dog VII (puppy).

Day 1. Inoculated with 2 c.c. of defibrinated heart blood.

11. No parasites found up to this time.

12. Again inoculated with 5 c.c. of defibrinated heart blood.

19. No parasites found up to this time.

20. Reinoculated with 6 c.c. of defibrinated heart blood.

22. A few parasites observed in the blood. Treatment begun : 1 grain of methylene blue administered in the form of a pill daily.

24. Found dead with many parasites in the blood.

*Result.* No evidence that treatment had any effect.



*Beta-naphthylamine.*

## 8. Dog VIII (puppy).

Day 1. Inoculated with 8 c.c. of defibrinated heart blood.

7. A few parasites found in the blood. 5 c.c. of a 3% solution of Beta-naphthylamine given subcutaneously.

8. Dog found dead.

*Control dog* (puppy) died with numerous parasites in its blood on the 6th day.

*Result.* Treated dog died 8th day and control dog on the 6th day.

## 9. Dog IX (puppy).

Day 1. Inoculated with 4 c.c. of defibrinated heart blood.

4. Few parasites found in the blood. Received 5 c.c. of a 3% solution of Beta-naphthylamine subcutaneously.

5. Received 5 c.c. of 3% solution of Beta-naphthylamine.

6. Not treated.

7. Found dead.

*Control dog* (puppy) died with numerous parasites in its blood on the 5th day.

*Result.* Treated dog died on the 7th day and the control dog on the 5th day.

*Atoxyl.*

In the experiments carried out by Gonder (1908, p. 301) the dogs were infected by means of intraperitoneal inoculations of blood containing *Piroplasma canis*. Three dogs served as controls while six dogs received treatment. One of the control dogs was treated after parasites appeared in its blood (control to experiments V and VI). We have not experimented with this drug but have condensed Gonder's descriptions in such a manner as to make them easily comparable with our own observations.

I. Dog "2" (1·25 years old) treated with atoxyl before inoculation. The dog received subcutaneous injections of a 1% solution of atoxyl every two days during two weeks, the dose being gradually raised from 5 c.c. to 10 c.c. (The total amount of the drug injected was not stated.)

Day 1. Inoculated with 3 c.c. of blood.

3. A few parasites found in the blood.

4. Many parasites in the blood. Haemoglobinuria. The condition grew steadily worse and

14. the dog died of piroplasmosis.

II. Dog "3" (7 months old) treated with atoxyl before and after inoculation. It was treated in the same way as dog I receiving 3—5 c.c. of a 1% solution of atoxyl on 6 days (45 c.c. of the solution in all).

- Day 1. Inoculated with 3 c.c. of piroplasma blood.
- 4. Parasites found in the blood.
- 5. Died of heart failure, parasites found in the organs at autopsy.

*Control* to experiments I and II : dog (2·5 months old) inoculated with 2·5 c.c. of piroplasma blood. A few parasites found in the blood on the 3rd day ; many on the 4th day, haemoglobinuria. Found dead on the 6th day.

*Result.* The atoxyl treated dogs died on the 14th and 5th days respectively after inoculation, the control dog on the 6th day.

III. Dog "6" (1·5 years old) treated with atoxyl before and after treatment. It received subcutaneous injections of a 1% solution of atoxyl every second day, the dose increasing gradually from 4 to 9 c.c. before the dog was inoculated. (79 c.c. of the drug were administered.)

- Day 1. Inoculated with 3 c.c. of piroplasma blood.
- 3. Received 10 c.c. of atoxyl.
- 4. Parasites observed. Haemoglobinuria.
- 5. Many parasites in the blood. 12 c.c. of atoxyl given.
- 7. Received 13 c.c. of atoxyl.
- 9.       "     14       "
- 11.       "     12       "
- 13.       "     9       "
- 14. Dog died. Many parasites in the organs at autopsy. Jaundice.

IV. Dog "5" (about 6 months old) treated with atoxyl from the time it was inoculated.

- Day 1. Inoculated with 3 c.c. of piroplasma blood. Received 4 c.c. of a 2% solution of atoxyl. (Total amount given during illness 13·5 c.c.)
- 3. Received 5 c.c. of atoxyl solution.
- 4. A few parasites found.
- 5. Many parasites in the blood. Haemoglobinuria. 4·5 c.c. of atoxyl given.
- 6. Dog killed, had symptoms of rabies ; advanced piroplasmosis.

*Control* to experiments III and IV ; dog (about 2 years old) inoculated with 3 c.c. of piroplasma blood. A few parasites appeared on the 3rd day and were found in the blood on the 10th day (intermittently). The dog recovered completely.

*Result.* Atoxyl treated dog, III, died on the 14th day, whilst dog IV was killed on the 6th day. The untreated control dog recovered completely.

V. Dog "8" (2 years old) treated with atoxyl from the time it was inoculated.

- Day 1. Inoculated with 3 c.c. of piroplasma blood. Treatment : received 8 c.c. of a 1% solution of atoxyl 3 hours before inoculation.
- 3. Received 10 c.c. of a 1% solution of atoxyl. Many parasites in the blood. Haemoglobinuria.
- 6. Received 6 c.c. of a 2% solution of atoxyl.
- 8. Received 8 c.c. of a 2% solution of atoxyl. (Total 22 c.c. of a 2% solution.)
- 9. Dog killed. Heavy infection.

VI. Dog "9" (2 years old) treated with atoxyl before and after inoculation. The treatment before inoculation lasted three weeks during which period the dog received 1% atoxyl solution in doses increasing from 2 to 12 c.c. After inoculation it received 2% solution, the total amount given being equal to 53 c.c. of a 2% solution.

Day 1. Inoculated with 3.5 c.c. of piroplasma blood. 14 c.c. of a 1% atoxyl solution was given on this and the day following.

4. Received 4 c.c. of 2% atoxyl solution.
5. Received 4 c.c. of 2% atoxyl solution. Parasites first observed in the blood.
6. Received 4 c.c. of 2% atoxyl solution. Parasites numerous. Haemoglobinuria.
7. Received 4.5 c.c. of 2% atoxyl solution.
8. Fewer parasites.
9. " " Received 4.5 c.c. of atoxyl 2% solution.
10. Dog very weak. Received 4 c.c. of atoxyl 2% solution.
13. Dog too weak to stand. Received 3 c.c. of atoxyl 2% solution.
- 14 and 15. Few parasites found.
16. Dog died. Death due to collapse and piroplasmosis.

*Control* to experiments V and VI: old dog inoculated with 4 c.c. of piroplasma blood. Parasites first found on the 4th day. This dog was afterwards treated with atoxyl every two days, the dose being raised from 4 to 8 c.c. (In all an amount equal to 70 c.c. of a 2% solution of atoxyl was given.)

- Day 5. Parasites first found. Haemoglobinuria.
10. Dog appeared better. Haemoglobinuria.
  12. Haemoglobinuria continues.
  18. " " Very few parasites.
  22. No parasites found in the blood.
  23. Dog appears well.
  25. Dog killed. Autopsy: spleen large, kidneys inflamed, and many parasites found in kidney smears.

*Result.* Atoxyl treated dog (V) was killed on the 9th day, and was found to be suffering from a heavy infection. Dog VI died from piroplasmosis on the 16th day. The control dog had apparently almost recovered on the 25th day. (Although considered as a control by Gonder this dog received more atoxyl than any of the other animals.)

Gonder's experiments show that atoxyl does not favourably influence piroplasmosis in dogs. It does not retard the appearance of the parasites or delay the progress of the disease. In fact the symptoms appear to be aggravated. The drug by itself produces similar effects to piroplasmosis, haemoglobinuria, bloody stools, great inflammation of the kidneys, and death due to cardiac failure (see Uhlenhuth, Hübener and Worthen, 1908, p. 277), so that satisfactory results could scarcely be expected.

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## NOTES ON NAGANA AND ON SOME HAEMATOOA OBSERVED DURING MY TRAVELS.

By HERBERT E. DURHAM.

### I. NAGANA.

SEVERAL observations on the disease caused by the *Trypanosoma brucei*, which were made after the publication of the paper by Kanthack, myself and Blandford<sup>1</sup>, have not yet been published, and since the points have not attracted the attention of other writers, it seems worth while to place them on record.

#### *Longevity of guinea-pigs suffering from Nagana.*

It was stated in the above cited paper that the longest period of survival, which till then had been observed in guinea-pigs, was 183 days. A still smaller maximum was observed by Laveran and Mesnil<sup>2</sup>, namely only 61 days.

When the virus was first received from South Africa, the experiments with guinea-pigs at first led us to believe that this animal was refractory. The guinea-pigs which had been inoculated and brought to England survived and did not show any signs of infection. Similar failure occurred with the earlier inoculations, which were performed by us with blood from the successfully infected dog which was delivered to us<sup>3</sup>. Further experiment however showed that the guinea-pig is not refractory, and many observers have had no difficulty in maintaining the strain by means of this animal.

The following observations are of interest, as showing that guinea-pigs may possess a considerable degree of resistance to the trypanosome:—

Two guinea-pigs ("A" and "B") were inoculated with blood of

<sup>1</sup> *Proc. Roy. Soc.*, vol. XLIV. 1898, p. 100, and *Hyg. Rundschau*, vol. VIII. p. 1185.

<sup>2</sup> *Trypanosomes et Trypanosomiasis*, Paris 1904.

<sup>3</sup> The syringe was used for all our earlier inoculations, later we used a surgical needle merely wetted with infective blood.

an infected horse on 3. v. 1897. Thereafter, though their blood was examined frequently, and though their temperatures showed occasional irregular rises, no trypanosomes were found in their blood. It may be presumed either that the infecting blood was at fault, or that they were resistant individuals. The sequel is somewhat in favour of the latter view. It may be noted here, that on the 43rd day after this first inoculation, rats were injected with the blood of each guinea-pig with negative result.

Guinea-pig "A" was reinoculated by the late Dr Kanthack on 10. II. 98 with blood of a guinea-pig containing active trypanosomes. Its blood was examined at intervals, and from 6. III. to 8. VI. 98 the presence of the trypanosome was noted. From that day onward trypanosomes were absent, so that on 24. XI. 98, this guinea-pig was again inoculated, the blood of an infected rat being used. The period covered was already more than nine months since the inoculation which had shown itself to be successful. Seventeen days after this third inoculation the blood was free from parasites, but by 24. II. 99 the trypanosome had become common, and the animal seemed to be ill. Eventually it died on 3. III. 99 or 99 days after the last inoculation. At the autopsy, the lymphatic glands were found to be exceptionally enlarged for a guinea-pig, the spleen was also much more enlarged than usual; in fact the appearances recalled those which obtain in the rat.

The further notes on guinea-pig "B" have unfortunately been lost.

Observations on another pair of guinea-pigs ("C" and "D") may be cited; these were first inoculated by Dr Kanthack on 22. II. 98 from an infected guinea-pig.

Guinea-pig "C" showed that it had been successfully infected, as trypanosomes were present in its blood 62 days later; eventually it died of the infection on the 102nd day.

Guinea-pig "D" is of greater interest. Trypanosomata were discovered in its blood 44 days after inoculation and again on 15. IV. and 25. IV. 98. Subsequent examination twice or thrice a month failed to reveal parasites. On 24. XI. 98 it was again inoculated, this time from a rat. Unfortunately on 20. II. 99 it was given another inoculation. On 8. III. 99 and again on 21. III. its blood was crowded with trypanosomes. Death occurred three days later. Counting from the original infection, the animal survived no less than 13 months, whilst the survival after the first reinoculation was 120 days; it may be noted that this infection was done from the same rat that served in the case of "A." It would seem then that although individual guinea-pigs may show

a considerable resistance to infection with the nagana trypanosome, yet they are not solidly immunised by apparent recovery from one attack.

*Relation of Birds to Nagana.*

In our joint report the fact is recorded that we were unable to infect pigeons with nagana; this has been confirmed by other writers, for instance Laveran and Mesnil.

It seemed possible that other kinds of birds might have a different reaction, and having obtained a kestrel (*Falco tinnunculus*), I inoculated it in the breast with citrated rat's blood, which swarmed with Nagana trypanosomes. The injection, about 2 c.c. in bulk, was given on 10. XI. 98. Twenty days later (1. XII.) the breast was punctured, and two rats were inoculated with the blood that was obtained, although no recognisable trypanosomes were to be seen. One of these rats died on the 12th day with trypanosomes in its blood and great enlargement of the spleen; its death, however, was complicated with a septic inflammation of the pleura. The second rat died on the 13th day, apparently of uncomplicated nagana, its blood was teeming with the parasites and the chief enlargement of the lymphatic glands corresponded with the site of injection (right thigh).

On 11. XII. the blood of the kestrel was again examined both locally from the breast and from the foot, but nothing that could be recognised as trypanosomal was found. Two more rats were injected with blood from the breast on this day, 31 days after the original inoculation of the bird. Both these rats succumbed to nagana; one was killed on the 11th day with teeming masses of trypanosomes in its blood; the other died on the 16th day. In both the chief glandular enlargement was in the right inguinal region, corresponding to the inoculation in the thigh.

On 24. II. 99 two more rats were inoculated, but they died after 24 and 48 hours of septic mischief.

On 6. III. 99 two more rats were injected with blood from the bird's breast. One of these died 35 days later without signs of infection by nagana, the other survived.

A reinoculation of the bird was performed on 25. IV. subcutaneously in the breast with about 1 c.c. of highly infected citrated rat's blood. On the next day only bird's and rat's red blood corpuscles, haemoglobin crystals and leucocytes could be found in a sample removed for inspection. Fifteen days later a rat was inoculated with blood from the breast, the result was negative.

On 16. VI. 99 nearly the whole of the blood (about 5 c.c.) of a highly infected rat was injected subcutaneously as before into the breast of the kestrel. On the next day a few active trypanosomes together with clumped masses of dark crinkled rat red corpuscles, leucocytes and granular matter were seen in a sample removed. A rat inoculated with this sample died in ten days of nagana. The bird was again examined locally three days after the injection, no trace of haematozoa or of rat blood corpuscles could be found; leucocytes however were abundant. Two rats inoculated died in 12 and 17 days of typical nagana.

On 30. VI. a rat was inoculated with negative results.

The kestrel remained in apparently good health. Owing to its fractious nature and the danger of injuring it, its temperature was not taken regularly, one day it was noted to be 41.7° C.; this is in accordance with the well known high temperature of birds.

In order to control this experiment with the kestrel, a *pigeon* was treated in exactly the same manner and contemporaneously. The dose given amounted to nearly 10 c.c. of citrated rat's blood (there were 2,500,000 trypanosomes per cubic millimetre in the undiluted blood).

Rats were inoculated on each of the four following days; only one of these died, death being within 48 hours apparently due to sepsis.

On 10. XI. 98 or 59 days after the first injection, a second inoculation of highly infective rat's blood was given. Rats were inoculated at various periods, all with negative result. The temperature record of the pigeon was rather higher than that of the kestrel, viz. 42.5° C.

Until actual observations have been made on birds of prey in nagana infested regions, it is only possible to say that they might conceivably be carriers of infection. Koch's observation that *Glossina* will feed on crocodiles is of interest in this connexion.

#### *Remarks on the Pathogenic Action of Trypanosoma brucei.*

If the course of the disease as it occurs in rats is compared with that which obtains in the rabbit, it is clear that the trypanosomal form of the parasite, as such in the circulating blood, has but a small share in the determination of illness. Thus a rat may have several hundred thousand trypanosomes in each cubic millimetre of its circulating blood, without showing grave signs of its rapidly approaching death. The same is true of guinea-pigs, for I have watched a buck which was most zealous in its attentions to a doe, a few minutes after counting 500,000 trypanosomes per cubic millimetre in its blood; moreover the animal's



weight record showed a continuous steady gain in weight since the inoculation.

On the other hand, progressive wasting and local swellings make a rabbit into a truly melancholy sight long before death supervenes, yet even with the aid of the centrifuge, the presence of trypanosomes may not be discoverable, until within a few days of death. The following example may be cited: a rabbit (2650 grams) was infected with a needle wetted with infective blood, 28 days later the weight had fallen to 1440 grams, the nose and eyelids were much swollen and obstructed. It was killed and its tissues searched for the presence of trypanosomes. The blood revealed none, in fact the only place where the parasites were found was in the rib marrow, and there they were quite sparse. Bone marrow in other regions was examined with negative result. It might be thought that the presence of the parasite in so important a tissue as the rib marrow might be significant to explain the disease, were it not that the trypanosome occurs in great numbers in the similar marrow of the rat. It is, perhaps, not inapt to remark that in malaria, the mature or almost mature parasites do not assert their presence with the marked disturbance of health, which is caused by the shower of a young brood through the system.

In animals, especially when they are near unto death from nagana disease, the blood shows marked morphological changes; the red blood corpuscles are reduced to about half their proper number, the leucocytes may be considerably increased, and nucleated red corpuscles are to be found. In my experience nucleated red corpuscles appear more abundantly in the rat and mouse than in other animals. Thus on one occasion the blood count of a rat, which was moribund, gave the high figure, 4,500 nucleated red corpuscles and 28,500 leucocytes per cubic millimetre. At this late stage of the disease the red corpuscles do not run well into rouleaux but tend to aggregate into small clumps. This clumping of the red corpuscles is evidently due to an alteration in the plasma or serum, for healthy corpuscles are caused to aggregate in a similar manner by mixing them with the diseased serum.

In some respects, nagana recalls the condition known as pernicious anaemia in man; and it may be noted that in 1897, I inoculated several rats with blood taken from patients suffering from this complaint. Cases are rare and I was not successful in trying from a case, which had not had arsenic administered therapeutically. At the same time several rats were injected with blood from patients suffering

from various kinds of leuchaemia. None of these animals showed any deviation from health or signs of parasites.

There is another blood change in nagana which on the whole is best seen in the rat when in an advanced stage of the disease. This change is manifested by a change of colour of the blood from the brilliant scarlet to a dull purplish or chocolate colour. The contrast between the healthy blood and the diseased blood is very striking when it is kept from coagulating by means of citrate, and can be recognised at a distance of some yards. In the clotted condition the phenomenon is likewise apparent, though hardly so marked. This dull coloured blood may be shaken with air, or allowed to stand for a week or more without developing the full red of normal oxyhaemoglobin. By the addition of the diseased blood to healthy blood the colour of the mixture is more or less dulled, so that the presence of healthy haemoglobin is not capable of causing a discharge of the abnormal colour. Examination with the spectroscope showed the bands of oxyhaemoglobin. Dr F. G. Hopkins kindly examined a specimen and considered that the oxyhaemoglobin bands did not appear quite normal. Dr Haldane has kindly drawn my attention to some observations<sup>1</sup> on the blood colour in poisoning with nitrobenzol derivatives. It appears that some species exhibit such changes whilst others do not do so; thus nitrobenzol causes no blood colour change in mice, though it does in dogs and cats. With dinitrobenzol the blood of the rabbit becomes chocolate coloured; this seems in part due to the presence of methaemoglobin but some other abnormal pigment is also probably at work.

Considerations such as these lead to the thought that though we now know much of the life-history and morphology of the parasites, this knowledge has taught us little, if indeed anything, concerning the disease itself. The same may be said of malaria in which morphology has not availed to advance our knowledge beyond diagnostic and preventive measures, the real nature of the disease and its symptoms yet remain to be unfolded. Another blood change, which may be mentioned as a matter requiring further elucidation, was noted in nagana-cachectic rabbits and consisted in the comparative resistance to haemolysis by the quillaia saponins. These saponins (sapotoxin and quillaic acid) are capable of haemolysing red blood corpuscles in high degree, the extent varying in different species of animals. If 1% dilutions of normal blood are treated with the glucosides it is found

<sup>1</sup> Haldane, Makgill and Mavrogordato, *Journ. of Physiology*, xxi. Nitrobenzol and dinitrobenzol, p. 184.

that small additions of serum of normal blood have some sheltering or delaying action. Normal cat or dog serum has a greater delaying effect than normal rabbit serum, but it was found that the serum of rabbits in the highly cachectic condition of the later stages of nagana had a still greater effect. von Dungern attributes variation in this response to haemolysis to the amount of lecithin substances in the case of normal animals; such may also be the cause in nagana.

*Summary.*

1. Some cases of remarkable resistance to nagana infection are recorded.
2. Although such birds as pigeons are unable to harbour the *trypanosoma brucei*, the kestrel is able to do so.
3. Attention is drawn to certain changes which are brought about by the trypanosome infection and the need for more precise chemical investigation of these haematozoal diseases.

*Notes on blood parasites observed in Christmas Island (Straits Settlements) and in the Malay Peninsula.*

Christmas Island possesses quite a number of peculiar species in its fauna, and it is regrettable that observations were not made before animals had been imported to this isolated station, as well as that my own notes are so incomplete.

*Rats.* There are three species of rat (1) the large *Mus nativitatis* had already become rare about the settlement at the time of my visits and I was unable to obtain either a freshly killed or a living specimen although a reward was offered.

(2) *Mus macleari*. A number of these rats was obtained and examined, of these nine were collected about the settlement; all were males and two of them had abundant trypanosomes in their blood. The trypanosome appeared morphologically like the *T. lewisi* of English rats.

Two other *M. macleari* were caught on the top of Phosphate Hill, some three or four miles from the settlement, one was a male the other a female and both were free from infection. Another was taken about half way up the hill and showed abundant trypanosomes in its blood;

the coat of this specimen was very infested with fleas<sup>1</sup>, but there were none of the small ticks which were found on other specimens.

It is, perhaps, noteworthy that the spleen of this rat was very markedly enlarged, as also were the superficial lymphatic glands. This animal was a rover and possibly had acquired its infection from imported *Mus rattus*. The presence of more lesion than occurs usually with *T. lewisi* infections led to a working hypothesis that the annihilation of native rats by imported ship rats may be due to the introduction of trypanosomes, which, finding a "virgin soil" to work upon, cause fatal epidemics. Unfortunately all the later specimens that were examined proved to be free of infection.

Out of 12 specimens examined 3 (or 25 %) were affected with trypanosomiasis.

(3) *Mus rattus*. The specimens were identified at the British Museum, they varied much in colour from the so-called "grey" to black. The manager of the Phosphate Company, Captain Vincent, informed me that these rats were first introduced to the Island in December 1899 by the SS. *Hindustan* in a cargo of hay; they had multiplied to very great numbers at the time of my visit 1901-1902, but apparently they remained about the settlement. Altogether 13 of these rats were examined haematologically, and six of them were found to be harbouring a trypanosome of similar appearance to *T. lewisi*. In regard to this parasite, the presumption is, that it was introduced.

A species of biting fly, much like our *Stomoxys calcitrans*, was also very prevalent about the settlement and might spread infection.

*Bats*. Two specimens of the large *Pteropus natalis* were examined and both had infection with a small malaria-like parasite in their red corpuscles. Sporulation was not taking place at the time of examination. The coats of the bats were full of a louse-like parasite. Other means of spread of the infection is to be found in the mosquitoes. I took three species on the Island, *Culex alis* (nov. sp. Theobald) being a new one, *C. fatigans* (only few specimens seen) and *Stegomyia scutellaris* which was very common and active during the day; the activity of the latter during the sleeping period of the bats would favour their attack.

*Birds*. The small ground pigeon, *Chalcophaps natalis*, was examined

<sup>1</sup> *Loemopsylla nesiotis* sp. nov. (Jordan and Rothschild, *Parasitology*, vol. 1. p. 1. 1906). The Hon. N. C. Rothschild has kindly informed me that all the specimens I obtained from *M. macleayi* were of this peculiar species, so that an interchange of fleas from *M. rattus* is not proven.

once and found to be severely infested with a halteridium-like parasite, the specimen was taken near the settlement. Here again there is doubt whether the parasite existed as an original inhabitant, for a number of carrier pigeons had been introduced.

*Carpophaga whartoni* was not examined, and *Zosterops natalis* was examined but showed no blood parasite.

Of the three blood parasites, in rat, bat and pigeon, those of the rat and pigeon have probably been introduced, whilst that of the bat seems likely to have been an old standing native occurrence.

Whilst on the topic of animal infections it may be mentioned that I made a number of blood examinations on birds at Kuala Lumpur Federated Malay States, often with the kind help of Dr J. D. Gimlette. The ordinary pigeons showed heavy infection with *Halteridium*, so also did two pet specimens of a small green parrot. The sparrows and a small species of pie were all free of infection, though several of each were examined.

The observations on nagana were carried on with the aid of the Tsetse fly Committee of the Royal Society, and the other notes were made during the Beriberi expedition of the London School of Tropical Medicine.

NOTE ON THE POLYMORPHISM OF  
*TRYPANOSOMA GAMBIENSE*.

By E. A. MINCHIN, M.A.

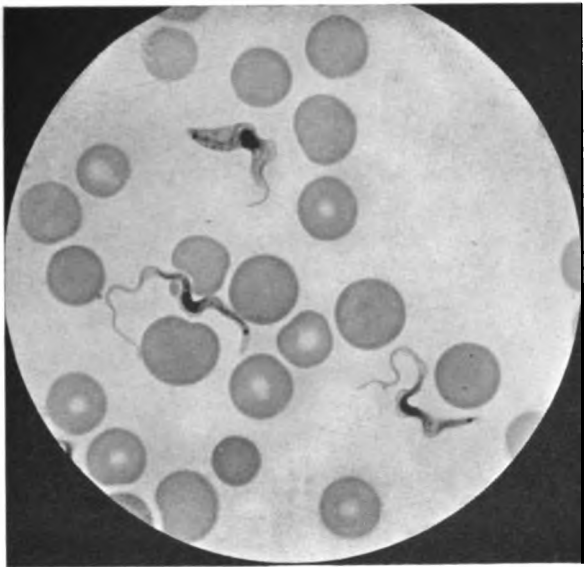
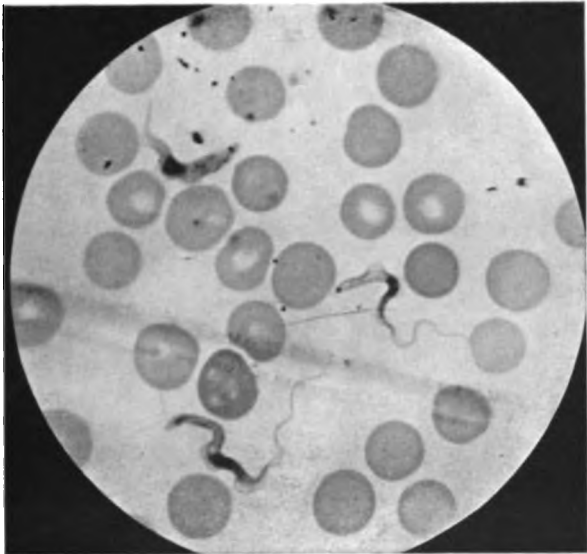
Plate XVII.

IN a memoir recently published, Salvin-Moore and Breinl<sup>1</sup> state that when *Trypanosoma gambiense* is examined in the blood "it does not seem possible to detect any true dimorphism or trimorphism." "The three forms often described and alluded to as distinct, consequently appear to be arbitrarily chosen examples in a continuous series of dimensions." It has always seemed to me very remarkable that the great differences in form and structure, no less than in size, between the slender, ordinary, and stumpy forms of *T. gambiense*, differences noted by all competent observers, should have been denied by two authors who claim for their methods of technique a superiority over those employed by all other investigators.

In a preparation of *T. gambiense* from the blood of a rat, which was made by my friend and assistant Dr J. D. Thomson for the cabinet of the Protozoological Laboratory of the Lister Institute, the three typical forms of the trypanosome were found to be very distinctly differentiated. Dr Thomson found and marked two fields in which the three forms occurred in close proximity, so that it was possible to photograph them. The blood-smear was fixed wet with osmic vapour, stained with Giemsa's stain, and mounted in Canada balsam. The two photographs which are reproduced here were taken at a magnification of 1000 diameters by my friend Dr D. J. Reid.

It can be seen clearly from the photographs that the difference between the three forms of *T. gambiense* is by no means one merely of size. The slender form is of great length and has a very long free flagellum. On the other hand the stumpy form is short and its

<sup>1</sup> *Annals of Tropical Medicine and Parasitology*, I. (1907), pp. 450, 451; compare also II. (1908), p. 212.







flagellum is also very short, especially the free portion. The ordinary form, which is much the commonest on the slide, is more or less intermediate between the two extreme forms. There is nothing new in these statements, and it seems almost necessary to apologize for restating such obvious facts; it is to be hoped that it will not be necessary to do so again.

As regards the significance of these forms, I incline to the opinion that the two extremes, slender and stumpy, represent sexual forms differentiated in opposite directions from a neutral type. This view receives support from the observation made by me, that after about 12 hours in the tsetse-fly only slender and stout forms are to be found. Nevertheless the question of the meaning of this well-marked trimorphism cannot be regarded as definitely settled in the present state of our knowledge.

## ON THE STRUCTURE OF "HALLER'S ORGAN" IN THE IXODOIDEA.

BY PROF. G. H. F. NUTTALL, F.R.S., W. F. COOPER, B.A.  
AND L. E. ROBINSON, A.R.C.Sc.

(Plate XVIII and one Text Figure.)

IN the year 1881, G. Haller published a description of the sensory organ of ticks which has since borne his name; he was apparently so much impressed by its remarkable similarity in structure to the auditory organs of many Crustacea, that he assumed it to be an organ of hearing, and even went so far as to describe the presence of otoliths within one of the cavities of the organ. Since that time, other zoologists appear to have given little attention to this structure, the majority of those who have had occasion to mention it, contenting themselves with a brief resumé of Haller's observations and a reference to his paper.

The first to question the accuracy of Haller's interpretation of the function of the organ, was Lahille (1905), who published the results of numerous experiments made on living specimens of the South American Cattle-tick, *Boophilus annulatus* var. *microplus*. The conclusion which he drew from his observations was, that the function of Haller's organ is olfactory and not auditory. Lahille's observations concern only the function of the organ; he gives no anatomical description. Batelli (1891) calls attention to the fact that ticks frequently move their first pair of legs (bearing Haller's organ) in the manner of the antennae of insects, and suggests that the organs serve as a means of perceiving, at a distance, the presence of the host-animals which are so indispensable to their existence. He also points out the remarkable resemblance of Haller's organ to the sensory organs described by F. Dahl (1885), occurring in *Pachygnatha listeri*, but, after an examination of Dahl's description and figures, we think that the points of resemblance are somewhat remote. Other observers have alluded to the antenna-like movements of the first pair of legs in ticks (vide Wheler (1899, 1900), Hunter and Hooker

(1907), and Hooker (1908)). Regarding the structure of the organ, Lewis (1892) in a few cursory remarks on the subject, adds nothing to the information to be obtained from Haller's account. Martin (1895, p. 273, see Plate XIV, fig. 8) observed three vesicles on leg 1, in *Amblyomma quantini*: the median vesicle was described as spherical in shape and contained a small compact mass—apparently an otolith; the other two vesicles contained hairs only. Dönitz (1907) summarises, in three paragraphs, our knowledge of the structure and function of Haller's organ, up to that time.

In the course of our work on the Ixodoidea, we have investigated the anatomical structure of the organ in every genus, and we have come to the conclusion that Haller's description is erroneous in many respects, hence the present communication.

Throughout the entire super-family, the principal features of the structure of Haller's organ are sufficiently constant, that, for present purposes, a description of the organ as exhibited in *Haemaphysalis punctata*, after making due allowance for generic and specific differences in details, will suffice as a guide to all.

Haller's organ is a minute cavity or vesicle (diameter about  $65\ \mu$ ) containing sensory hairs and associated with specially modified hypodermal tissues which lie immediately beneath it. It is borne on the dorsal surface of the terminal article (tarsus) of the first pair of legs; Haller's statement that it is borne "auf der Bauchfläche der Extremität" is incorrect. It bulges down into the interior of the leg, its surface being flush with the surrounding parts, and is formed entirely of chitin which is continuous with the chitinous cuticle of the leg. The superficial part or roof of the vesicle is formed of thin transparent chitin, through which a minute slit-like pore opens (Fig. 1, p. 240), establishing communication between the interior of the vesicle and the external air<sup>1</sup>. In lateral view (Plate XVIII), as usually seen in mounted specimens of the tarsus,

<sup>1</sup> Knowing well the difficulty of correctly interpreting the real nature of minute structures formed of highly refractive chitin, especially in the case of a minute and thin-lipped pore, we have succeeded in demonstrating the patency of this opening in a fairly convincing manner. A leg of the first pair was snipped off a living tick, with scissors, and immersed in a syrupy solution of Canada balsam on a cover-glass: this was inverted over a small glass chamber mounted on a glass slip, in the manner of a hanging-drop preparation. A small lateral tube opening into the chamber made it possible to connect the apparatus with an air-pump, the excess of balsam on the cover-glass forming an efficient luting to form an air-tight fitting. The apparatus was placed on the stage of the microscope, and after sharply focussing the pore of Haller's organ, the air was slowly exhausted. In every case, immediately exhaustion commenced, a bubble of air was seen to exude from the pore.

the cavity of the vesicle is seen to be divided by folds or thickenings of its chitinous lining, into two chambers—a smaller superficial chamber from which the above-mentioned pore opens to the exterior, and a larger chamber, the floor of which, on the side towards the proximal end of the tarsus, is raised into a number of shallow conical papillae (Plate XVIII, Fig. 1), each of which bears a sensory hair. The sensory hairs are straight and stiff and protrude across the cavity of the larger chamber, their points being directed towards the irregular and indefinite opening between the two chambers. The sensory hairs are 20—25 $\mu$  in length

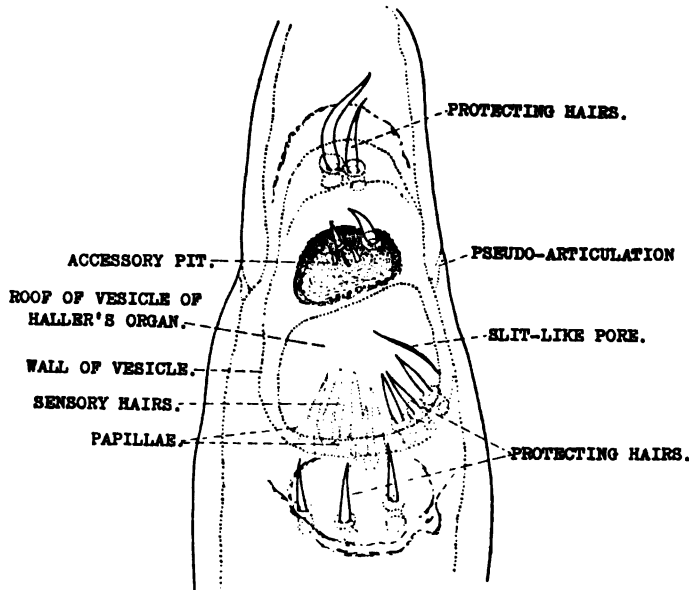


Fig. 1. Haller's organ as seen from the dorsal aspect.  $\times 330$ . (L. E. R.)

and differ in their appearance from the other hairs on the body and appendages of the tick: the internal cavity of each is comparatively large, the bounding walls are unusually thin, and there is no constriction at the base, the contour of the shaft of the hair merging into that of the basal papilla without any perceptible line of demarcation.

With regard to the structure of the hypodermis underlying Haller's organ, we have been limited to a study of sections, the material for which was preserved by methods unsuitable for the determination of the structure of delicate cellular elements, and until new supplies of living

material are forthcoming, we are unable to complete our work on the histology of the soft parts. From the few observations which it has been possible to make, it appears that the hypodermal cells immediately beneath the sensory hairs are large and flask-shaped; each consists of a sac of finely granular cytoplasm surrounding a large central cavity or vacuole which communicates directly with the hollow interior of the hair. We have succeeded in identifying nerve fibrils running towards the bases of the sensory hairs, but cannot, at present, define their relationships with the modified hypodermal cells.

Closely associated with the vesicle of Haller's organ and included in Haller's description as an intrinsic part, is a pit-like depression which we have termed the *accessory pit* (Plate XVIII, Fig. 1): it lies a little distance beyond the distal side of the main vesicle, is widely open to the exterior, and is furnished on its floor with a number of short stiff hairs, some of which protrude beyond the mouth of the pit. This structure appears to have been considered as a small counterpart of the chief vesicle, but its appearance, together with the fact that the hairs which it contains are quite different in character from those of the latter, does not support this view. The surface of the leg on both the proximal and distal sides of Haller's organ bears a cluster of stiff hairs, the function of which is, presumably, protective (Text fig. 1).

As already stated, Haller alluded to the presence of an otolith in the cavity of the organ and a representation of such a body is clearly shown in his figure: we have diligently searched for such bodies in all our preparations but have failed to detect their presence and have no hesitation in denying their existence.

In conclusion, we are compelled to doubt that the function of Haller's organ is auditory. Haller based his interpretation on the presence of a supposed tympanic membrane, of otoliths, and the resemblance to the auditory organs of certain Crustacea. No one, as far as we know, has attempted any experimental proof of an auditory function. On the other hand, the structure of the organ, the peculiar antenna-like movements of the first pair of legs, and the results of Lahille's experiments are all strongly in favour of the assumption that Haller's organ is olfactory in function.

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## DESCRIPTION OF PLATE XVIII.

- Fig. 1. *Haemaphysalis punctata*. Lateral view of left tarsus of female.  $\times 440$ . (L.E.R.) The figure is slightly schematised and represents the vesicle of Haller's organ and the accessory pit in optical section.
- Fig. 2. *Haemaphysalis punctata*. ♀. Photomicrograph of Haller's organ from the side, showing the pore, accessory pit and part of the chitinous fold which differentiates the smaller chamber from the larger.  $\times 150$ .
- Fig. 3. As in Fig. 2. This photograph gives some slight indication of the slit-like character of the pore.  $\times 400$ .

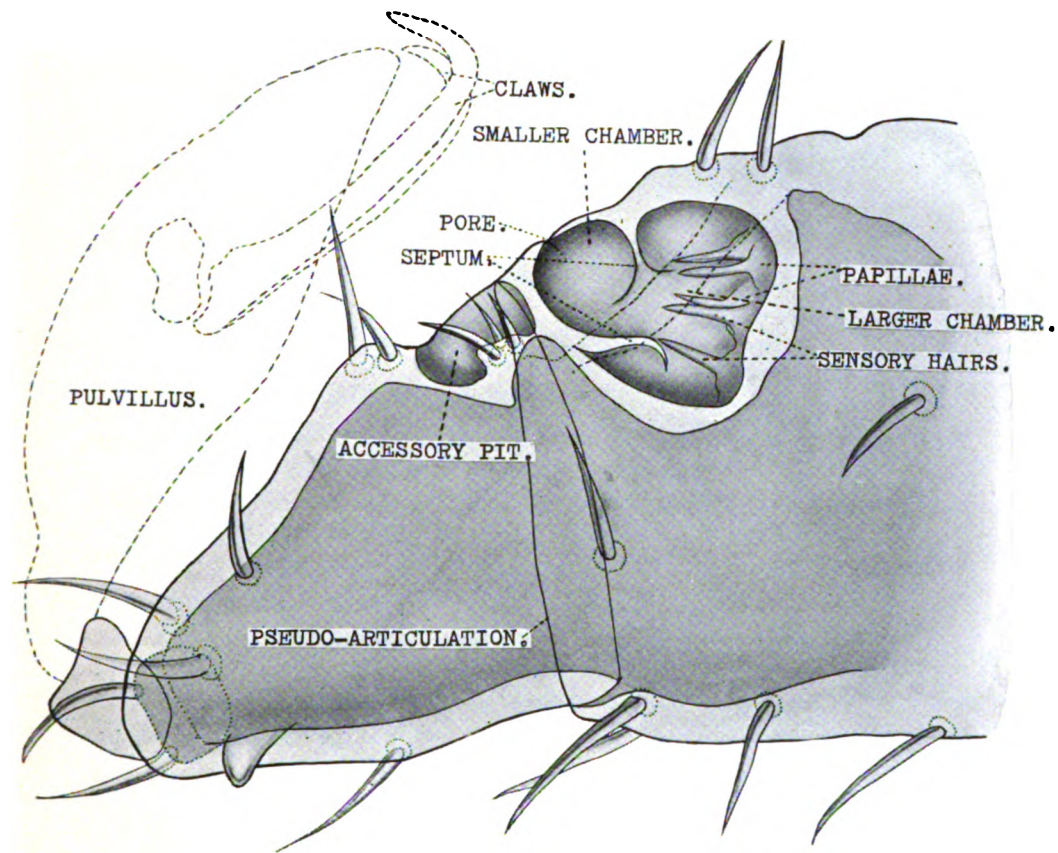


Fig. 1.



Fig. 2.



Fig. 3.





## THE DEVELOPMENT OF PIROPLASMA CANIS IN CULTURE.

By GEORGE H. F. NUTTALL AND G. S. GRAHAM-SMITH.

Plate XIX and 1 Text Figure.

IN the course of their investigations upon different species of *Piroplasma* numerous observers have kept infected blood in the ice-chest, or at room temperature, for various periods of time prior to the inoculation of animals. On the evidence afforded by microscopic examination alone some observers have concluded that the parasites are capable of considerable multiplication in defibrinated blood kept *in vitro*. Under these conditions many corpuscles undergo haemolysis and in consequence the parasites appear to be more numerous. We believe that this source of error explains the conclusions arrived at by Lignières and others with regard to the multiplication of *P. bovis* in extravascular blood (see p. 253).

It is only within the last few years that systematic attempts have been made to *cultivate* the different species of *Piroplasma*. We have previously referred (1905, *Journ. of Hygiene*, v. 245) to the negative results which followed the attempts of Nocard and Motas (1902, pp. 274—275) to cultivate *P. canis*, although they established the fact that the parasites remained alive and virulent in blood which had been preserved in the dark and cold for 25 days. Kinoshita (1907, p. 111) has kept the parasite alive for 31 days on ice. In an earlier paper we have described the forms of parasites observed in defibrinated dog's blood up to 48 hours after its removal from the animal.

### *Kleine's observations.*

Kleine (1906, pp. 10—15) states that most of his attempts to cultivate *P. canis in vitro* proved negative until he adopted Robert Koch's suggestion and studied the early changes which take place in cultures made by diluting defibrinated piroplasma blood with salt solution. Kleine infected young dogs by the intraperitoneal injection of about

10 c.c of defibrinated piroplasma blood. When the parasites became numerous in the blood of the infected dogs (they usually died on the third or fourth day) he bled the animals to death under chloroform, defibrinated their blood and added 0.5 c.c. of blood to 0.5 c.c. of salt solution. A series of 20 such cultures in test tubes were made at one time and kept at 27° C.

After the lapse of 18 hours Kleine decanted the clear supernatant fluid from the tubes and upon examining the deposit found that it contained bodies corresponding in form to those described by Koch (1905, 1906) in piroplasma infected ticks. Many of the parasites occurred in the form of clubs with about six rays protruding from the broader portion, whilst the tapering extremity also bore two or more radial processes. The parasites appeared peculiarly rigid, but, on closer examination in drop culture, amoeboid movements could be detected, the parasites becoming slowly rounded or elongated, whilst the length and number of the radial processes changed. These changes were already observable after eight hours, but were most marked after 18 hours.

After 48 hours some parasites had attained a length of 14  $\mu$  and a width of 4  $\mu$ , the rays at the broad end measuring 9  $\mu$ , those at the opposite end measuring up to 19  $\mu$  in length. These large parasites occurred together with parasites of normal size. When stained by Giemsa's method the large forms with radii showed a large chromatin mass at the end of the club, and usually a secondary chromatin mass at the tapering extremity. Kleine states that the rays appear blue, unless intensely stained when they take on a red colour. When the parasites were numerous in the blood, masses of radiate bodies occurred, the tapering extremities of the parasites converging to a common centre. When the parasites were very numerous the development of these large forms appeared to be inhibited. At times Kleine observed "fused forms" such as Koch has described in ticks, sausage shaped bodies with radii protruding from the extremities and containing large masses of chromatin at the ends of the sausage and secondary masses of chromatin situated about midway along their length.

After two days at 27° C. the parasites appeared on the whole larger than normal. In forms from early cultures both chromatin masses stain in the same way, but in those derived from cultures two days old the chromatin mass within the clubs retains the peculiar black-red appearance, whilst the larger chromatin mass surrounded by radii appears more lightly stained. After 2—3 days the parasites cease to

exhibit radii, they become rounded and an achromatic zone appears round the dark chromatin mass. The number of degenerating forms increases after the third day. The process of multiplication in defibrinated and diluted blood could not be observed, and no multiplication took place when the developmental forms were transferred to fresh normal or diluted blood.

Kleine thinks that in canine piroplasmosis the parasite usually lies upon the red blood corpuscle. We have elsewhere stated our reasons (*Journ. Hygiene*, VI. 636; VII. 250) for regarding these parasites as being intracorpuseular, and see no reason for changing our opinion<sup>1</sup>. Kleine also considers that the cultivation forms with radii are usually epicorpuseular, but here again we hold a contrary opinion. Owing to its extreme delicacy and lack of staining power the corpuseular envelope may perhaps have escaped his notice in many instances. Occasionally the corpuseular membrane cannot be demonstrated and then the parasite appears to be free.

#### *The writers' experiments.*

In our attempts to cultivate *P. canis* under artificial conditions we have made use of several different methods and media, which are described in the following pages. Under most of these conditions the parasites rapidly degenerated without exhibiting any signs of multiplication, or changes of form suggesting further development. These negative experiments (A—L) are first described.

In the last series of experiments (M) however certain large forms with long radiating processes were encountered, similar to those described by Kleine and Koch.

A. In order to ascertain whether the parasites would enter and multiply in normal dogs' corpuscles kept outside the body under artificial conditions the following experiments were made. Blood-stained serum taken shortly after death from the heart of a dog dead of piroplasmosis was centrifugalised, and drawn up into capillary pipettes.

<sup>1</sup> Kleine's statement and that of Kinoshita (1907, p. 309) regarding the epicorpuseular position of *Piroplasma canis* appears to be based upon deceptive appearances in stained preparations. Kleine's paper is illustrated by excellent coloured figures and microphotographs which show appearances essentially similar to those we have observed. We have not however seen large agglomerations of radiate bodies such as he figures on Plate IV, fig. 14, and Plate V, fig. 12.

Kinoshita apparently confined his attention to the study of stained preparations of *P. canis*. He has consequently advanced various hypotheses regarding the development of the parasite which we do not consider to be justified.

At the end of an hour the clear fluid contained some free motile pyriform bodies. Some of this fluid was now added to defibrinated normal dog's blood and unstained preparations of the mixture thus obtained were examined. Although free swimming parasites were kept under observation, with one exception which we have previously described (*Journ. of Hygiene*, 1906, Vol. VI. p. 632), none were seen to enter fresh corpuscles.

In other experiments some of this fluid was mixed with normal dog's corpuscles suspended in sodium citrate solution, and the mixture kept in the dark at room temperature for 20 hours. At the end of this time fresh preparations were examined and motile parasites seen, but none were found in corpuscles. Stained specimens confirmed the observations made on living preparations.

Similar experiments were carried out with serum obtained from blood which was allowed to clot, with the same results.

B. Dog's serum containing motile pyriform bodies was added in small quantities to tubes containing rabbits' blood corpuscles suspended in 4% sodium citrate solution. Examinations of fresh and stained preparations showed that the parasites had not entered the corpuscles.

C. The following experiments were undertaken to determine whether the parasites would live and multiply in the serum of infected animals. Shortly after death blood-stained serum from the heart was drawn up into tubes and centrifugalised. Two hours later the clear fluid showed many rounded parasites and a few actively motile pyriform bodies. After 17 hours a few rounded forms were found, but no motile parasites.

Serum taken from coagulated blood was examined in the same way. This also showed some rounded forms and a few motile pyriform bodies after two hours, but no motile forms after 22 hours.

D. Several experiments were made with *defibrinated blood* taken from the heart immediately after death. Some samples were kept in sealed tubes, and others in tubes plugged with cotton wool. Some of these tubes were kept in the ice chest, and others at room temperature. Examinations of fresh and stained preparations were made at various times with samples taken from the various layers into which the fluid separated. In all 15 experiments were carried out and the examinations were made at times varying between 17 hours and 69 days. Motile pyriform bodies were noticed up to 17 hours, but not later, and non-motile pyriform bodies were seen up to the third day. After this time all the free parasites seen were either rounded or in various stages

of degeneration. The majority of the intracorpuseular forms soon became rounded, but a few of the pyriform ones retained their shape for many days. Occasionally large numbers of rounded parasites were found within leucocytes. (See Plate XIII, fig. 3, *Journ. of Hygiene*, Vol. VI. 1906.)

In some cases the red blood corpuscles retained their normal shape up to 35 days.

E. Eleven experiments were made with blood agar in the hope of obtaining cultivation forms. The blood agar was made by mixing equal parts of defibrinated rabbit's blood with melted agar at 55° C. and was prepared one hour before use. Small quantities of fresh defibrinated heart's blood of dogs dead of the disease were placed in the tubes, which were cultivated at various temperatures. Examinations made at various times revealed only a few degenerated parasites.

F. Defibrinated dog's blood added to various quantities of 2% sodium citrate solution was kept under the same conditions and examined in the same way as in experiment D. Seven experiments were made, the times of the examinations varying between 28 hours and 72 days. Even after 28 hours the majority of free forms and intracorpuseular parasites had become rounded. After this time very few free parasites were seen. In one experiment a few forms with well marked slender radiating processes like those described later (p. 252) were seen. In some cases the red blood corpuscles were found well preserved up to 53 days.

G. Five similar experiments were made by adding defibrinated blood to slightly acid 2% sodium citrate solution with the same results.

H. In three experiments defibrinated blood was added to 4% sodium citrate solution and the cultures examined at various times between 13 hours and 23 days. With very few exceptions both the intracorpuseular and free parasites were found to be rounded. Many of the red blood corpuscles retained their shape up to the 23rd day.

I. Two experiments with normal saline solution containing 4% sodium citrate gave similar results.

J. In six experiments defibrinated blood was added to 25% potassium oxalate solution and specimens were examined between 36 hours and 62 days. The same changes were noted as in experiment F. In some cases the red blood corpuscles retained their normal shapes up to 60 days.

K. Six experiments with slightly acid potassium oxalate solution gave the same results.

L. Following the method of Miyajima (1907) (see p. 255) numerous cultures were made by mixing defibrinated blood and ordinary nutrient broth in proportions varying between 1—5 and 1—10. These were kept for various periods at temperatures ranging between 18° C. and 35° C. Though the red blood corpuscles were well preserved the parasites rapidly became rounded and lost their motility. No developmental forms like those described by Miyajima were seen and none with radiating processes.

M. In the series of experiments about to be described certain very large irregular intracorpuseular forms with radiating spike-like processes were occasionally met with, apparently identical with the cultural forms described by Kleine, and resembling the free parasites observed by Koch in the early stage of infection in the tick.

In the following description these are spoken of as forms with radiating processes and the processes as radii.

In all the following experiments the cultures were made by adding a quantity (about 0.5 c.c.) of blood, defibrinated by shaking with glass beads for 20 minutes, to an equal quantity of 0.6% or 0.8% salt solution or to a physiological saline solution (hereinafter referred to as "P" solution) with the following composition:

Sodium chloride	...	...	0.95%
Potassium chloride	...	...	0.025%
Calcium chloride	...	...	0.02%
Sodium hyd. carbonate	...	...	0.15%
Dextrose	...	...	0.1%
Distilled water	...	...	100 c.c.

*Dog I.* The cultures were made in 0.6% salt solution. Some of these cultures were kept in test tubes plugged with cotton wool, and others in Petri dishes, slightly tilted, at various temperatures (14° C., 16° C., 20° C. and 32° C.). Examinations of fresh and stained preparations were made at various times. At 14° C. after 20 hours a few forms showed short blunt processes. At 16° C. no forms with radiating processes were seen. At 20° C. after 16 hours one form with short radii was found, but at 32° C. several parasites showing radii were observed after 24 hours. These experiments seemed to indicate that a temperature between 20° C. and 32° C. was the most suitable for the development of forms with radiating processes, and consequently in the subsequent experiments the cultures were kept at 24° C.—26° C.

*Dog II.* Cultures were made in 0.6% salt solution and were kept

at 26° C. After 20 hours a few forms such as are depicted in Plate XIX, figs. 2—4, were seen in corpuscles which had lost their haemoglobin, but none with true radii were found.

*Dog III.* Several cultures were prepared in 0.8% salt solution and examined at various times, but no forms of interest were met with.

*Dog IV.* Cultures were made in 0.6% and 0.8% salt solution and kept at 24° C. for 27 hours. Several preparations made from the surfaces of the corpuscular layers of various cultures all showed numerous parasites with radiating processes from which the specimens shown in Plate XIX were drawn.

Preparations made from the other layers and the supernatant fluid showed very few if any parasites with radii.

*Dog V.* Ten cultures were made in 0.6% salt solution and kept at 24° C. Examinations at 17 and 25 hours were all negative as regards parasites with radiating processes. After 41 hours a single form with true radii was found.

*Dog VI.* A very complete series of cultures and examinations were made in this case. Cultures in 0.6% salt solution were kept at 24° C. Preparations made after 15 hours and after 25 hours showed no parasites with radii. Examinations after 43 hours, however, showed numerous large forms and some with true radii. These forms were still present a few hours later.

No forms of interest were noted in cultures made with 0.8% salt solution after 25 hours. After 43 hours several large forms were seen and one with true radii. Five hours later these forms were more numerous.

In "P" solution no interesting forms were seen after 15 and 24 hours. After 43 hours three forms with radiating processes were noted, and a few after 48 hours.

*Dog VII.* In this case all cultures were kept at 24° C. and were left absolutely undisturbed until they were examined.

In 0.6% salt solution after 28 hours parasites were numerous and a few forms with radii were seen. After 27 hours' cultivation no obvious changes were noticed, but after 73 hours many forms with well marked radiating processes were seen.

In 0.8% salt solution a few forms with radii were seen after 28 hours, and after 47 hours they were numerous, but after 73 hours their numbers had decreased.

In "P" solution no forms with radii were seen after 28, 47 and 73 hours' cultivation.

*Dog VIII.* In this case all the cultures were kept at 24° C. In 0·6% salt solution after 18 hours large forms were numerous, some of which had radiating processes.

Examinations made after 32 and 48 hours' cultivation showed many large forms, a few of which possessed well marked radii.

In 0·8% salt solution and in "P" solution large forms were seen, but none with true radii.

*Dog IX.* Cultures were made in 0·8% salt solution and were kept at 24° C. After 30 hours many large pyriform bodies were seen, a few of which showed radiating processes, but after 44 hours both the large forms and those with radii were less numerous. Similar cultures in 0·6% salt solution and in "P" solution showed no forms with radiating processes.

*Dogs X, XI, XII, and XIII.* Similar experiments were made with blood derived from these animals, but no forms with radii were seen.

*Dog XIV.* Cultures made in 0·8% salt solution and kept at 24° C. for 26 hours showed numerous forms with well marked radii. Motility was observed in some of them.

*Dogs XV, XVI, XVII, XVIII, and XIX.* Similar experiments were made with the blood of these animals but no forms with true radii were seen.

From the foregoing account it can be seen that in some experiments numerous forms with radiating processes were encountered, whilst in others, apparently conducted under exactly similar conditions, none were found. We have been entirely unable to find any cause for these differences in the behaviour of the cultures.

The differences apparently do not depend on the age of the animal from which the blood was taken, the height of the fever, or the rapidity with which it developed. Nor do they appear to depend on whether the temperature was falling or stationary, or on the period at which the blood was taken either in regard to the date of inoculation, or to the time at which the fever appeared. The following table summarises the data on which these statements are based.

The forms with radiating processes were found most commonly in cultures made in 0·6% salt solution, and were most frequently obtained from the uppermost part of the layer of blood corpuscles. Although it appears to be necessary to use small quantities of fluid, probably in order to ensure the presence of a sufficient oxygen supply, we did not find that better results were obtained in shallow tilted dishes, with extremely shallow layers of fluid. Cultures which were left absolutely



at rest gave better results than those which were occasionally or continuously shaken, or through which a current of air was intermittently passed. The times at which the forms with radiating processes were most numerous varied in the successful cultures. In dog VIII they were numerous after 17 hours' cultivation, and were fewer after 44 hours. In most cases however they were most numerous on the second day. After that time they usually decreased in numbers.

*Positive experiments.*

Dog	Age	Temperature		Condition when blood taken	Date after inoculation	Days after fever commenced
		At death	Mode of rise			
I.	puppy	103 °F.	—	stationary	3 days	2 days
IV.	"	103·8	slow	falling	9 "	4 "
V.	"	105·8	"	stationary	7 "	5 "
VI.	"	106	"	"	6 "	2 "
VII.	"	104	"	"	4 "	0 "
VIII.	"	103·8	"	"	6 "	2 "
IX.	"	103·8	rapid	falling	9 "	1 "
XIV.	"	103	slow	"	6 "	2 "

*Negative experiments.*

II.	puppy	101·4° F.	slow	falling	12 days	6 days
III.	"	103	rapid	stationary	10 "	2 "
X.	"	—	?	"	5 "	1 "
XI.	2 years	104	rapid	"	4 "	2 "
XII.	old	103·8	"	"	5 "	1 "
XIII.	puppy	103·6	"	"	5 "	1 "
XV.	"	103·6	"	"	6 "	1 "
XVI.	"	102·2	very little	"	7 "	3 "
XVII.	"	104	rapid	"	4 "	1 "
XVIII.	"	103·6	slow	"	5 "	2 "
XIX.	"	—	—	—	—	—

*Description of the cultivation forms.*

In cultures made in 0·6% and 0·8% salt solution forms of the parasite may often be seen, which apparently are not degeneration forms, and which are never met with in the circulating blood or in the organs. The majority of these are large intracorpuseular forms. In very many cases the infected corpuscle has lost its haemoglobin, and its contour can only be made out with difficulty by following the faint line marking the rim of the collapsed and often much enlarged corpuscle. Occasionally no corpuscular remains can be defined and the large parasite appears to be free. Nevertheless we are inclined to think that

most of these apparently free forms are really intracorpuseular, and that failure to find the corpuseular envelope is due to its extreme delicacy and lack of staining power.

The least differentiated forms are large definitely intracorpuseular parasites, such as are figured in Plate XIX, figs. 2—4. Some of these show two well defined masses of chromatin (fig. 1) while others, usually of irregular shape, show three or more masses (figs. 2, 3, 4). Very rarely extremely large forms are seen with several masses of chromatin apparently connected together by thin strands of chromatin (fig. 21). Other examples of intracorpuseular parasites of the same general type show minute, delicate, radiating processes (previously described as radii) projecting beyond the parasites, and usually originating in the neighbourhood of a chromatin mass (figs. 5, 6). Others show much longer processes, frequently long enough to reach the margin of the corpusele (figs. 7, 8, 9). These processes, which are often extremely delicate, especially at their distal extremities, vary greatly in number, sometimes being almost too numerous to count (fig. 12).

Occasionally several large parasites of this type are seen in one corpusele (fig. 20).

In yet more remarkable forms some of the processes cause small projections or even considerable distortions of the corpuseular envelope (figs. 9, 11, 13), and occasionally may even perforate the envelope and project for a considerable distance beyond it (figs. 13—16). Similar forms (figs. 17, 18, 19), apparently free, are occasionally seen.

In living preparations all intracorpuseular parasites, except some of the rounded forms, show slight movements, probably of a molecular nature, since small masses of detritus with active dancing movements are often seen within the envelopes of the collapsed corpuseles.

On several occasions we have kept under observation living examples of the forms with long processes and have noticed changes of shape accompanied by the very slow protrusion and retraction of the radiating processes. The accompanying figure illustrates the changes noticed in one parasite (A) during 50 minutes' observation, and in another (B) during a period of five minutes.

Owing to the uncertainty of the cultural methods and the difficulty of keeping living parasites under observation for long periods we have been unable to follow the development of these forms. Possibly the forms with numerous processes develop from forms with many chromatin masses (fig. 21) and the latter by fusion from multiple intracorpuseular forms such as are illustrated in fig. 20.

In the absence of observations on living parasites the interpretation of the appearances seen in stained preparations is extremely hazardous<sup>1</sup> and we do not feel justified in offering any conjectures as to the origin and significance of the bodies we have described.

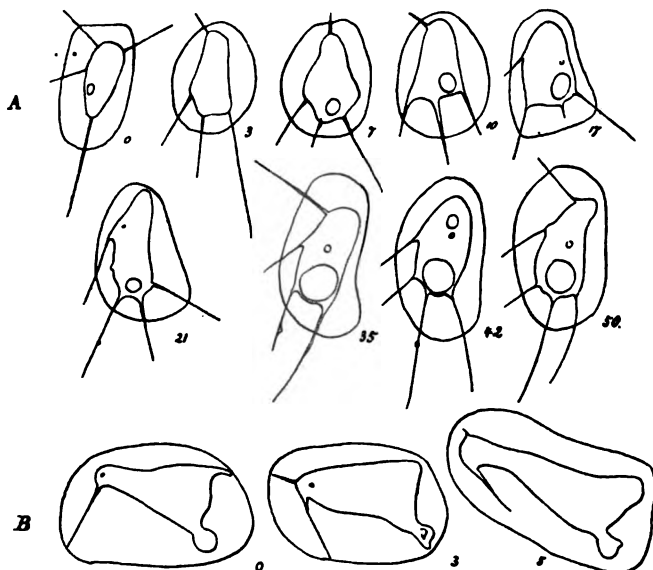


Fig. 1.

#### APPENDIX.

##### 1. Attempts to cultivate the other species of *Piroplasma*.

Lignières (cited by Chauvelot, 1904, p. 11) using blood containing many parasites did not succeed in cultivating *P. bovis* in different kinds of broth, gelatin, agar, or on potato, serum, amniotic fluid, or aqueous humor, whether the cultures were exposed to the air, kept in an atmosphere of carbon dioxide, or hydrogen, or kept *in vacuo*. He also obtained negative results with blood kept in collodion sacs. As a culture medium he also tried serum rich in haemoglobin obtained from diseased cattle, adding to it 0.25 c.c. of blood containing many parasites,

<sup>1</sup> Breinl and Hindle (1908) have recently asserted that *P. canis* multiplies in the blood in several different ways. Observations on the forms assumed by the parasite in the living blood during multiplication, which can be studied without great difficulty, do not lend any support to their hypotheses, which are based on the evidence derived from wet films stained by Breinl's method.

which had shown "marked multiplication" at the bottom of tubes containing blood. After 15 days, according to Lignières, the parasites in haemoglobin serum showed marked multiplication and a third subculture gave even better results than did the second. All the culture forms were spherical and he states they contained one to four germs which leave the parent cell, grow rapidly and often remain in pairs connected by a filament which cannot be demonstrated by staining. Lignières (1903, Pl. IV) illustrates such forms in a later publication but his deductions appear to be so largely hypothetical that we do not deem it necessary to consider them further.

Dschunkowsky and Luhs (1904) tried to cultivate the parasites in haemoglobin serum in test tubes. A grayish deposit, which could be shaken up, formed at the bottom of the tubes. They thought they observed evidence of great multiplication at the end of 10—20 days. The parasites also appeared to multiply in defibrinated blood both inside and outside the red blood corpuscles. The parasites were definitely motile and appeared to multiply up to 20—25 days both at room temperature and at 38° C. (It was supposed at the time that they were working with *Theileria parva*.)

#### *Piroplasma ovis.*

Motas (1904, p. 31) attempted to cultivate this parasite in blood, blood serum and haemoglobin serum at different temperatures. His results were all negative. He notes that the blood gradually loses its virulence when preserved at 15—18° C., but may remain virulent up to 10—14 days. The blood remains virulent for longer periods, up to 15—20 days, if maintained at lower temperatures, provided no bacterial growth takes place. If the tubes become contaminated virulence is only retained up to 5—10 days. The parasites did not live nearly so long when citrate or oxalate of potash were added to the blood. The virulence was not affected by an exposure of 12 hours to minus 5—6° C. or by exposure to 43—44° C. for the same time.

#### *Piroplasma equi.*

Theiler (1903, p. 99) states that he has on several occasions kept the blood of horses containing this parasite *in vitro*. The blood was placed in test tubes and kept at different temperatures, in the ice box, at room temperature, and in the incubator. In the incubator the parasites disappeared from the blood as soon as the corpuscles lost their

haemoglobin. When the blood was kept at room temperature they persisted longer. In blood kept in the ice box the parasites after 12 days appeared as distinct as in fresh blood, but in most cases had become spherical, and were situated at the edges of the infected blood corpuscles. Very exceptionally free parasites were encountered. Some of the corpuscles which contained parasites did not stain in the vicinity of the parasite, so that the latter appeared surrounded by a colourless zone. Theiler suggested that this zone might be due to the destruction of the haemoglobin by some excretion product of the parasite. Although he transplanted the parasites into fresh serum he was unable to observe any multiplication such as Lignières states he observed in the case of *P. bovis*. He regarded the parasites which stained well after 12 days *in vitro* as alive.

Bowhill (1905, p. 2) mixed infected horse blood with potassium citrate in a flask and kept it at room temperature. After 24 hours many circular and oval extra-corpuscular parasites arranged in irregular masses were seen. Stained by the Romanowsky method the chromatin and protoplasm stained red and blue respectively. On adding fresh serum to infected blood and keeping the latter at room temperature, at 29° C. or at 40° C. he was unable to detect any multiplication of the parasites, although on one or two occasions he observed amoeboid movement.

*Theileria parva* (= *Piroplasma parvum*).

We have elsewhere (ix., 1908, p. 516) stated our reasons for excluding this parasite from the genus *Piroplasma*, but since it belongs to an allied genus it is desirable to consider the cultivation experiments of Miyajima (1907, p. 84).

This observer, working in Japan, states that he tried to cultivate the parasite in blood agar, sodium citrate (acid and alkaline), beef extract, peptone water, calf-serum, normal saline solution, and common broth, to which media he added infected blood. He obtained positive results when he added *Theileria* blood to ordinary broth in the proportion of 1—5 to 1—10 and maintained the cultures at 20—30° C.<sup>1</sup>

"The development of the parasites in a successful culture takes place in the following manner: on the first day no motile form is seen;

<sup>1</sup> Miyajima states incidentally that he was able to cultivate *Tr. lewisi* under similar conditions. Although we have tried to cultivate this parasite on several occasions according to Miyajima's method we have not succeeded in doing so.

on the second, there can be observed a certain number of peculiar cells which occupy the upper layer of the sedimented corpuscles and which microscopically appear as a series of white dots. Very few motile forms resembling trypanosomata are visible in these cells on the third day of incubation, but thereafter the trypanosomata multiply vigorously and reach the maximum number between the tenth and fourteenth day."

"In a culture kept at room temperature, the trypanosomata remain motile until 45 days later, at this time most of them have undergone degeneration and globular cells with irregular granulation result."

When kept at 10—20° C. the trypanosomes were alive after a lapse of three months.

Miyajima states that he was able to maintain the parasites alive in subcultures as Novy and others had done with *Tr. lewisi*.

According to Miyajima *Theileria parva* therefore appears to develop into a trypanosome under cultural conditions in blood added to broth. Nine of the 21 native cattle examined showed *Theileria parva* and from the blood of seven out of the nine trypanosomes were obtained in cultures. The transplantation of a single loopful of blood was sufficient to secure a positive culture. The development described by Miyajima appears to be extremely rapid. After three days at 25° C. the diminutive *Theileria* is stated to have attained or exceeded the size of a red blood corpuscle, and to have become actively amoeboid. Very large vacuolated cells appear after 20 hours and curious crescentic bodies after 48 hours which give rise to typical flagellates. After 72 hours besides the nucleus and blepharoplast the flagellates exhibit an undulating membrane and can be seen to divide longitudinally, gradually giving rise to large colonies.

As controls Miyajima examined 200 cattle but *Theileria* were not found in blood films and trypanosomes did not develop in cultures.

Miyajima confirmed the observations which have been made in other countries that *Theileria* cannot be transmitted to clean animals by blood inoculations. On the other hand he found that two out of three clean calves inoculated with cultures containing the trypanosomes became infected in eight days with red-water. One of these animals gave a positive result with cultures (development of trypanosomes) 17 days before the appearance of *Theileria* in its blood upon microscopic examination. If Miyajima's observations are correct they are certainly most interesting and remarkable, but a certain amount of scepticism appears justified until they have been extended and confirmed by other workers. It will be remembered that Schaudinn (1904, p. 438, see

*Journ. Hygiene*, VI. p. 642) advanced the hypothesis that *P. bovis* undergoes development into a trypanosome, a statement which has gained no support from subsequent investigations. Should *Theileria* develop into a trypanosome we would have an additional ground for separating it, in the light of our present knowledge, from *Piroplasma*.

## 2. *Blood-platelets and haematozoa.*

In connection with these cultivation experiments Swingle's (1908) observations on the similarity between blood-platelets and certain haematozoa are of interest. He made cultures of normal sheep and other blood in a medium of the following composition: water 1000 c.c., sodium citrate 5 grms., sodium chloride 5 grms. made slightly acid with hydrochloric acid. One drop of fresh blood was added to 2 c.c. of the solution. For making stained preparations he placed a drop of the culture fluid on a slide and after it had evaporated down, but was not entirely dry, he dropped on some killing fluid such as Zenker's solution. The specimens were stained by various methods.

He remarks that "normal platelets resemble normal piroplasma forms in size and general shape. A nucleus, and refractive spots, probably vacuoles, can be seen" (p. 49). We cannot entirely agree with this statement, since in our own preparations we have seldom found any difficulty in distinguishing between blood-platelets and forms of the parasite.

Swingle's observations, which are quoted at length, on the cultivation forms of platelets are extremely interesting. "Cultures examined as quickly as possible after drawing the blood showed amoeboid and flagellate forms. A fact of great importance to the student of haematozoa is that the most of these forms are capable of movement. They seem to roll over, swing round, and often move for a distance equal to the diameter of two or three red corpuscles. Among the amoeboid types there were always present in great numbers platelets with few or several long, sharp, or sometimes blunt, pseudopodia. From the description and figures of Koch and Kleine one must conclude that these forms are very similar to, if not identical with, what they describe as developmental stages of *Piroplasma*. To be sure Koch found his stages in the stomach of the tick, but this does not disprove the statement, for just such forms were also found in the stomach of the sheep tick after sucking the blood of a sheep....In older cultures most of the platelets that had no pseudopodia were at the rim, either inside or just

outside of a transparent circle about the size or a little larger than the platelet. Those outside looked as if they had crawled out of a thin envelope. As to their significance, I can only say that they apparently were not degeneration forms, inasmuch as they were still active in their amoeboid movements. The more typically flagellate forms, those with a single flagellum, are perhaps of still more importance, because they so perfectly simulate real flagellates. Although they are found immediately after the introduction of the blood into sodium citrate solution, the 'flagellum' is generally quite rigid except at the very end, where it can be seen to vibrate. Notwithstanding this rigidity, they seem to move about, roll over, and swing round, these movements probably being the result of the vibrations at the tip of the flagellum. The most motile forms were found in a culture of human blood kept for the first six hours in an ice chest and after that at room temperature for 50 hours. Round or pear-shaped individuals with a flagellum measuring in some cases as much as  $20\ \mu$  were found in abundance. In the pear-shaped forms the flagellum is at the pointed end. It was very slender, in most cases appearing and moving very much like the flagellum of *Euglena*, often with lashings violent enough to move the red corpuscles on coming in contact with them. Instead of being smooth, in some instances the flagella had thickened, knotted portions, which bear a close resemblance to Kinoshita's description and drawings of the flagellates which he found. It is important to note that he found the best developed flagellate with a flagellum  $15\ \mu$  long in sodium citrate culture.

The various forms are often found grouped together in couplets, triplets, or in masses composed of as many as a hundred individuals. In this condition they retain their individual motion, rolling over and turning about. I have seen these masses stained with iron haematoxylin so that they had the exact appearance of Kleine's photograph."

Swingle says that he found such masses in the stomach contents of sheep ticks fed on sheep's blood. "To eliminate the possibility of confusing the platelets with the herpetomonadine flagellates, which are generally present in adult ticks, they were studied in young ticks before the latter had become infected."

"Flagellation of blood-platelets is not limited to sodium citrate culture, but may take place in other media."

"In stained preparations one often finds a most striking, yet doubtless merely coincident, resemblance in nuclear conditions to trypanosome forms. While nuclear dimorphism is not by any means to be



found in all blood-platelets, yet it is by no means rare. And when it does occur there is not such marked distinction between the nuclear masses as one sees in trypanosomes, but still as much distinction as many of Kinoshita's drawings would indicate for *Babesia*....Thus it would be an easy matter to mistake such blood-platelets for real flagellates having true nuclear dimorphism." "The flagella and pseudopodia stain like cytoplasm, and not like chromatin as in the trypanosomes."

Swingle concludes his paper with the following words: "It is not sufficient answer to the similarity I have shown to say that Kleine used defibrinated blood, and hence blood-platelets were not present in his solutions, for no one has demonstrated that the platelets are entirely removed by defibrination. Since blood-platelets in various culture media and in the stomach of the tick always develop flagella, move about, and manifest such a marked resemblance in form, size, and structure to *Babesia* and the Leishman-Donovan bodies, investigators must furnish criteria to differentiate between the flagellated platelets and the parasites. Until they have established their position by experiments with normal blood, the correctness of their results can be accepted only with some reserve. The evidence I have presented shows that neither are motion and flagellation exclusive characters of parasites nor will they differentiate them from blood-platelets. Each student will have to determine experimentally how to distinguish the two classes of structures."

As controls for our observations we have carried out a number of experiments by Swingle's method, using *defibrinated* blood from normal and infected dogs. Occasionally we have found platelets such as he has described, but the majority of cultures showed none. We do not think that the intracorpuseular forms of piroplasma with radiating processes could be mistaken for platelets, and up to the present we have not met with any "flagellated" platelets which could be mistaken for the extracorpuseular parasites.

## DESCRIPTION OF PLATE XIX.

Fig. 1. Large free pyriform parasite with two chromatin masses.

2, 3, 4. Large intracorpuseular parasites each with several chromatin masses.

5, 6. Large intracorpuseular parasites with short radii.

7. Small intracorpuseular parasite with several radii.

8, 9. Intracorpuseular parasites with long radii.

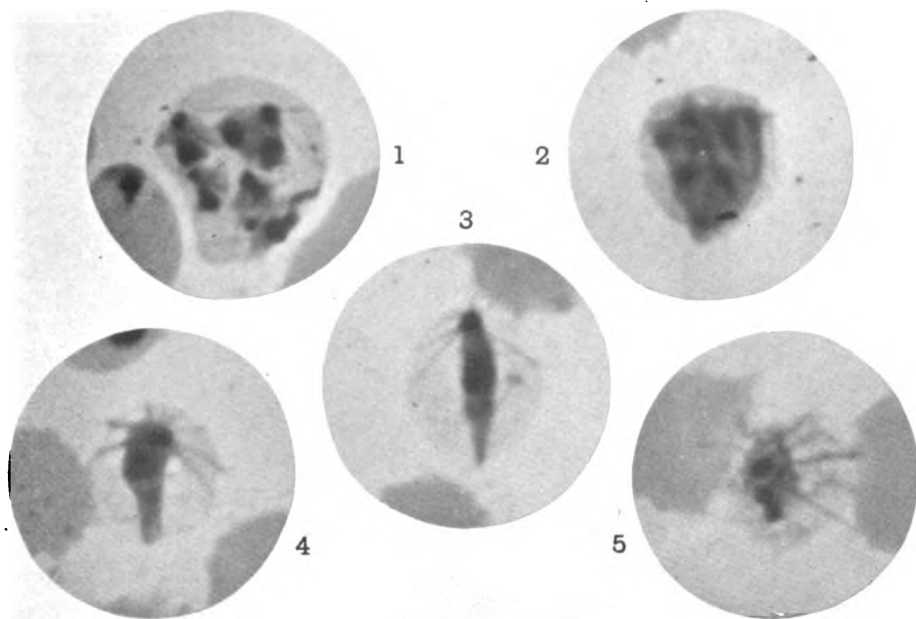
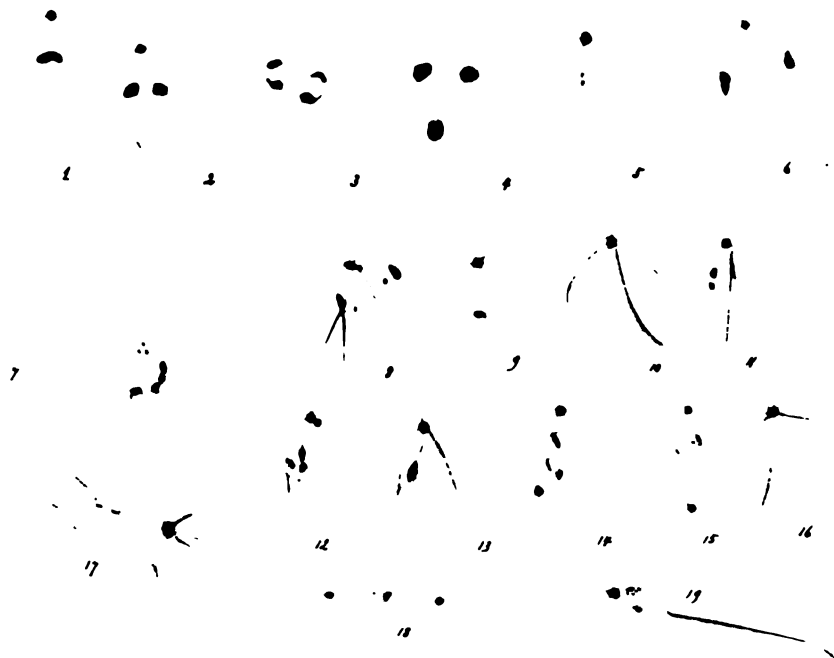
10-16. Intracorpuseular parasites with numerous radii, some of which apparently penetrate the corpuseular envelope.

Figs. 17-19. Large free parasites with long radii.

20. Photograph ( $\times 3000$ ) of corpuscle containing several parasites with short radii.
21. Photograph ( $\times 3000$ ) of corpuscle containing a large triangular parasite with several chromatin masses connected by thin strands.
- 22, 23. Photographs ( $\times 8000$ ) of corpuscles containing large pyriform parasites with several long radii projecting from their blunt extremities.
24. Photograph ( $\times 3000$ ) of corpuscle containing a large rounded parasite with numerous radii.

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# NOTE ON THE PREVALENCE OF INTESTINAL WORMS IN DOGS IN CAMBRIDGE.

BY GEORGE H. F. NUTTALL AND CYRIL STRICKLAND.

IN the course of investigations conducted in Cambridge upon dogs infected with *Piroplasma canis*, a number of animals have been incidentally examined for intestinal parasites. In an unbroken series of 24 dogs, which died (March—September 1907) from piroplasmosis, the animals were autopsied immediately after death and the intestine examined by slitting it open along its whole length. We briefly record the results of these examinations, for the reason that similar reports do not appear to have hitherto been published in this country. All of the dogs harboured worms. Only three species of worms were encountered: *Ascaris mystax*, *Dipylidium caninum* and *Taenia serrata*.

The parasites were distributed as follows:—

	No. of dogs har- bouring each species		
<i>A. mystax</i>	..	...	17
<i>D. caninum</i>	...	...	14
<i>T. serrata</i>	...	...	4

The species occurred either alone or associated with each other:

10 dogs harboured *A. mystax*.

5 " " *D. caninum*.

5 " " *A. mystax* and *D. caninum*.

2 " " *D. caninum* and *T. serrata*.

2 " " *A. mystax*, *D. caninum* and *T. serrata*.

The numbers of *A. mystax* varied from 1 to 113 per dog.

" " " *D. caninum* " " 1 to 102 " "

" " " *T. serrata* " " 1 to 8 " "

The total number of each species recovered from the 24 dogs was

*A. mystax* 296.

*D. caninum* 312.

*T. serrata* 17.

The following table contains details regarding the numbers and species of worms found in each of the dogs examined:—

No. of dog	Species of worm	No. of worms found	No. of dog	Species of worm	No. of worms found
1	<i>A. mystax</i>	4	13	<i>A. mystax</i>	3
2	<i>D. caninum</i>	1	14	<i>D. caninum</i>	1
	<i>T. serrata</i>	3	15	<i>D. caninum</i>	82
	<i>A. mystax</i>	2	16	<i>A. mystax</i>	2
3	<i>D. caninum</i>	2	17	<i>D. caninum</i>	30
	<i>T. serrata</i>	1		<i>A. mystax</i>	6
4	<i>A. mystax</i>	5		<i>D. caninum</i>	1
5	<i>A. mystax</i>	1	18	<i>A. mystax</i>	45
6	<i>A. mystax</i>	48		<i>T. serrata</i>	5
7	<i>A. mystax</i>	4	19	<i>D. caninum</i>	1
8	<i>D. caninum</i>	7	20	<i>D. caninum</i>	53
9	<i>D. caninum</i>	20		<i>A. mystax</i>	7
	<i>T. serrata</i>	8	21	<i>A. mystax</i>	5
10	<i>D. caninum</i>	7	22	<i>A. mystax</i>	31
	<i>A. mystax</i>	2		<i>A. mystax</i>	113
11	<i>A. mystax</i>	4	23	<i>D. caninum</i>	4
	<i>D. caninum</i>	1	24	<i>D. caninum</i>	102
12	<i>A. mystax</i>	14			

## A CAUSE OF APPENDICITIS AND OTHER INTESTINAL LESIONS IN MAN AND OTHER VERTEBRATES.

BY A. E. SHIPLEY, M.A., F.R.S., HON. D.SC. (PRINCETON),

*Fellow and Tutor of Christ's College, Cambridge, and Reader in Zoology in the University.*

### Observations on Birds.

5 OUR observations on a large number of recently dead or dying grouse impels us to believe that in many cases death is primarily caused by the presence of parasitic worms, either Cestodes or Nematodes in various parts of the alimentary canal.

### CESTODA.

Conspicuous amongst the entozoa of the Grouse is *Davainea urogalli* (Modeer), which in the grouse is only found in the small intestine. This is the tapeworm known to sportsmen and to keepers; it indeed frequently protrudes from the hinder end of the alimentary canal and sometimes trails like a pennant behind a bird that is flying. Besides this we have very frequently a second genus and species of Cestode the *Hymenolepis microps* (Diesing) which we have for the first time recorded from the grouse, this occurs in the duodenum with a third species *Davainea cesticillus* (Molin), but as we have only found this twice it may be neglected in a consideration of the effects of the cestode parasites upon the health of the birds.

In this enquiry I propose to confine myself to the action of entozoa on the wall of the alimentary canal and having given a short, preliminary account of what happens in the grouse to consider the evidence which is accumulating of injury done to the human intestine, caecum and appendix by the presence of entozoa.

*Davainea urogalli* (Modeer).

Almost all grouse contain *D. urogalli*, only a very small percentage being free from it. Although the body is large the head is extremely small but capable of expanding and contracting. Its average diameter may be put at about 0.16 mm. This head is provided with a protrusible rostellum which bears a double crown of very minute hooks, about  $6.7\ \mu$  in length. There are also four well-developed suckers each bearing a large number of hooks varying from  $6.6\ \mu$  down to half that size. In both cases the hooks are bent and are very sharply pointed, thus the whole forms an admirable weapon for injuring the mucous layer of the alimentary tract.

We have not as yet found that in the grouse *D. urogalli* has set up any very marked lesions or that it has caused very serious disease, but that at times it is capable of doing so seems most probable from analogy with a closely allied species which causes the so-called "nodular disease" of the intestine so fatal to poultry. This is caused by a closely allied tapeworm *D. echinobothrida*<sup>1</sup> (Méglin, 1880) which in many respects is very like *D. urogalli*. Piana (1880) has figured transverse sections of the intestinal wall of a fowl which was infested with this species<sup>2</sup> and he shows how the tapeworm heads burrow right through the mucous lining of the intestine, entirely breaking its continuity, and come to rest in large vesicles filled with an exudate in the muscular sheath of the wall just below the serosa.

Moore (1895) has given a full account of the disease associated with these nodules, which vary in size from being scarcely visible to having a diameter of 4 mm. Moore states that they are either circular or lenticular and over the larger ones the mucosa sloughs leaving small ulcerated depressions. These also contain an exudate, a greenish-yellow necrotic substance and surrounding this a thin layer of infiltrated tissue. The smaller nodules contain a more purulent substance. His sections showed, as did Piana's, that the heads of the tapeworms had penetrated the mucous membrane and were situated in different layers of the intestinal wall. Though more difficult to detect in the larger and therefore older nodules they were almost invariably

<sup>1</sup> Ransome (1905) regards this species as distinct from *D. tetragona*, a common parasite of fowls, partly on the ground that it causes disease and *D. tetragona* does not.

<sup>2</sup> He called them *Taenia botrioplitis* but it is the same species.



found in the smaller and younger swellings. In the earlier stages a cell-infiltration envelopes the head of the *Davainea* in the nodule. Moore dwells upon the wide prevalence of this disease and the chances of its being mistaken for tuberculous disease in fowls. He states that it "is highly probable that the total loss it occasions both from deaths and from the shrinkage of poultry products, due to the chronic course of the disease it produces, is very large."

*Hymenolepis microps* (Diesing).

We found the second tapeworm, which exists in any abundance in grouse, in September 1905. It had hitherto escaped the notice of the numerous observers who have for years been working at grouse disease. Its name is *Hymenolepis microps* (Diesing, 1850) and it lives in countless numbers in the duodenum, yet it is unrecognizable when alive. In this state the contents of the duodenum resembles a thick purée. If to this purée we add corrosive sublimate the tapeworms, which are so transparent when alive as to be invisible, slowly whiten and reveal themselves as countless fine opaque threads each with one end—the head end—sunk in the walls of the alimentary canal. *H. microps* is a very fine, fragile but long, worm attaining at times a length of 15 cms. and it consists of an enormous number of proglottides. The head like the head of *D. urogalli* has a rostellum and four suckers, but the rostellum is alone armed with hooks. These are very numerous, some  $16\mu$  in length, very sharply pointed and shaped like slightly curved bayonets. In transverse sections of the duodenal wall of a grouse infested with these tapeworms, one sees the head "nuzzling" down between the villi.

NEMATODA.

Passing to the thread—or round—worms which infest the alimentary tract of grouse, here again we find three species, one of which, *Syngamus trachealis* von Siebold, is so rare as to be negligible; another species *Trichosoma longicolle* Rudolphi is so difficult to see that all previous observers overlooked it, and we ourselves did not find it for months, although when once found we had little difficulty in finding it again and again; and a third species *Trichostrongylus pergracilis* (Cobbold) known to the older observers and the apparent cause of profound disease in the grouse.

*Trichosoma longicollis* Rudolphi.

*T. longicollis* lives in the duodenum and upper end of small intestine (jejunum), sometimes alone, sometimes associated with *H. microps*. They are not very evidently associated with grave disease, but when they are present they seem to cause a great destruction of the lining epithelium which even in birds just killed and quite warm is cast off in large clumps and masses. This form like its nearly *Trichocephalus trichiurus*<sup>1</sup> of the human intestine has an extraordinarily fine head and neck, hardly much greater in diameter than one of the epithelial cells lining the grouse's duodenum and this could easily, and we believe does at times, pierce the wall of the intestine and so let out bacteria, harmless enough in the alimentary tract but capable at times of exerting a pathogenic action when they reach the tissues of the intestinal wall or the peritoneal cavity.

*Trichostrongylus pergracilis* (Cobbold).

The second common Nematode of the digestive apparatus of the grouse is the *Trichostrongylus pergracilis* found in the caeca, and here it should be mentioned that the caeca form a large and very important part of the digestive apparatus in a grouse. Together they are at least as long as the whole of the rest of the alimentary tract, and in them absorption of the digested food takes place. When the caeca of the grouse contain a large number of the *T. pergracilis* its tissues undergo profound changes. The pathological changes associated with the presence of these worms are still the subjects of investigation but that the presence of the worms is intimately associated with grave disease, there can be no doubt.

As was the case with the *Trichosoma* of the duodenum so is it with the *Trichostrongylus* of the caeca. Both when alive are as transparent as a *Sagitta*. For a long time we could only detect them after the addition of some such reagent as corrosive sublimate when they became opaque and visible. Latterly Dr Wilson has devised a simpler method of verifying their presence. It is to press a thin film of the caecal contents between two microscopic slides and to hold this up to the light, then if any worms are present they stand out as transparent lines against the background of the semi-transparent chyme.

<sup>1</sup> = *Trichocephalus dispar* Rudolphi, 1801.

*Syngamus trachealis* von Siebold, 1836.

Another case of the continuity of the lining membrane of an organ being destroyed is that caused by the *Syngamus trachealis* found in the trachea of poultry and pheasants. This so-called red- or forked-worm pierces through the wall of the trachea and actually clenches the teeth with which its mouth is provided in the cartilaginous tracheal rings. So close is the attachment that the body of the worm will rupture if when once firmly fixed attempts are made to pull it away from the tracheal wall. If the trachea contains septic organisms and the cartilage were easily infected by them a more efficient inoculating medium could not be devised.

**Observations on other Vertebrates.**

Before passing on to consider the relation of the nematodes to the wall of the human intestine and caecum I should like to draw attention to one or two striking cases of lesions caused by thread-worms in other Vertebrates.

**THE HORSE.**

Such an instance is the *Sclerostoma equinum* so often found in the colon and caecum of the horse. This nematode pierces the mucosa until it reaches the capillary blood vessels and then engorges itself upon the horse's blood. The walls of the alimentary canal infested with this parasite are dotted with small reddish ulcers which heal sooner or later according to the nature of the bacteria which have access to them. When the bacteria are pathogenic the ulcers are formed at the place of the lesion and here various forms of microbes are found<sup>1</sup>. These ulcerations can according to Weinberg extend until they attain an area of 23 mm. x 8 mm. In them the mucosa and often the sub-mucosa is destroyed and a marked infiltration of leucocytes, amongst which many bacteria occur, takes place in the deeper layers of the sub-mucosa. In other cases the ulcers are replaced by small abscesses some of which attain a considerable size (85 mm. x 33 mm.); they contain a fluid but there is little infiltration of leucocytes. These ulcers are especially common in horses that are wasting away and are attributed by

<sup>1</sup> Faure and Marotel (1902). I have not been able to see this Paper but I am indebted for a summary of it to Weinberg's article (1907). From this I have taken many references and many statements.

Weinberg to an infection by some peculiarly toxic, anaerobic bacteria. The very severe mortality amongst horses due to the presence of *S. equinum* is thus accounted for. The question where do the bacteria come from has been advanced a stage further by the researches of Weinberg and Saeves. They have succeeded in withdrawing the contents of the intestine of 97 *Sclerostomes* taken from 25 horses. Thirty-three of these worms contained bacteria in the contents of their alimentary canal: *B. coli*, "*Enterococcus*," and a *Diplobacillus*, whilst cultures yielded *Streptococcus* and *Staphylococcus*. It is thus evident that the *Sclerostomes* can in many cases infect the pierced tissues not only by chance bacteria sticking to their exterior but also by the contents of their intestine should they escape.

#### MAN.

With reference to bacteria adhering to the outside of entozoa and by them conveyed to places where they become pathogenic it may be mentioned that Piana was the first to show the migration of *Cysticercus pisiformis* into the liver of the rabbit. Metchnikoff (1901) first drew the attention of medical men to the danger which the presence of entozoa in the human intestine entailed upon their hosts. In the Harben Lectures (1906), he relates instances in which attacks of appendicitis have been associated with the presence of *Oxyuris* and *Trichocephalus* in the alimentary canal. Guiart and Grimbert (1906), further consider the matter in some detail. They consider that entozoa, especially round-worms, act as inoculating needles, and play a part in the etiology of certain diseases of the wall of the alimentary tract and of the liver comparable to that played by certain Diptera and Ixodoidea in the diseases of the blood. The gravity of the disease set up has of course a definite relation to the virulence of the bacteria admitted to the deeper tissues, and the course of the disease runs on quite independent of whether the inoculating needle—the entozoon—has been removed from the intestine or not, but removal naturally stops further infection.

If we now consider in turn the effects that three of the commonest human nematodes, *Oxyuris vermicularis*, *Ascaris lumbricoides* and *Trichocephalus trichiurus*, have upon the walls of the places they live in we shall find that the part played by these entozoa is being daily better appreciated.

*Oxyuris vermicularis* (Linnaeus, 1767).

O. Seiffert (1908) draws attention to the lesions in the mucous membrane of the rectum caused by these very common worms and to the fact of the intestinal catarrh they frequently set up. Wagener (1904) found amongst the Peyer's patches of a hog, five years old, 15—20 small nodules which when investigated microscopically revealed the calcified remains of *Oxyuris* worms. He considered that the worms had penetrated into the follicles, formed ulcers there, and when the ulcers healed had undergone calcareous degeneration. Ruffer (1901) also records a number of tumours in the rectum of a man, the tumours varying in size from a pin's head to a walnut. The tumours contained ova of *Oxyuris*; since these could not have got there by themselves the probability is that they were laid *in situ* by some female which had penetrated the rectal wall. Fröhlich is quoted by Weinberg as describing a case in which he found 16 *Oxyuris*, all females, living surrounded by pus in a tumour in the peritoneum of a child of 11 years of age. Edens (1896) found the head of an *Oxyuris* in a nodule of a Peyer's patch in a child of seven years whose intestine presented the typical lesions of primary, intestinal tuberculosis. There are many more examples, but these seem to me sufficient to show that *Oxyuris* can and, not unfrequently, does perforate the wall of the alimentary canal.

*Relation of entozoa to Appendicitis in Man.*

The relations of this worm with appendicitis may now be considered. The worms live in the lower part of the small intestine, in the caecum and in the appendix vermiformis. When the eggs begin to develop in the fertilized female the worms leave the caecum and appendix and passing through the colon arrive at the rectum; here they may lay their eggs but most of them creep out of the body to lay them elsewhere.

Galli-Valerio (1903) has described a case of an appendix which had been perforated and which contained many Oxyurids, the tail of one of the male specimens was threaded through the mucosa and microscopic sections showed spaces resembling the perforation which were surrounded by an infiltrated zone infected with bacteria. Weinberg (1906, 1907) gives at length an account for which he is indebted to Dr Thevenard of a boy aged 11 years, who was after much suffering operated on for appendicitis. On examining the appendix about 1.5 cm. from the free end

a small nematode—which proved to be a female *Oxyuris*—was found threaded through the mucosa and so firmly fixed that light traction at either end failed to dislodge it. The appendix was congested and the congestion was most pronounced in the neighbourhood of the parasite. Microscopic study of sections showed that the worm had pierced through the mucosa, traversed a gland, and reached the vascular layer in the sub-mucosa. These sections also showed a very inflamed condition of these parts, the parasite was surrounded by polynuclear leucocytes and amongst them a large number of bacilli. There were also signs of lymphangitis around the same spot extending into the sub-serous layer and here the blood vessels were very congested. All these disturbances had their centre in the impacted *Oxyuris*.

Seiffert (1908) in his summary of the relation of entozoa to appendicitis quotes the following:—Still (1899) recognizes Oxyurids as the great cause of catarrhal affections in the appendix: Moty (1902) recognized Oxyurids as the cause of three of his cases of appendicitis: in Morkowitin's (1902) cases numerous Oxyurids were obviously the disposing factors to appendicitis: Ramstedt (1902) found a regular tangle of *Oxyuris* in an extirpated appendix and believed that they had set up the inflammation: Oppe (1903) found *Oxyuris* in six appendices and thought that a "Wurmkur" should be considered in cases of appendicitis—this is especially indicated when examination of the faeces reveals the ova of *Oxyuris* or *Ascaris*; *Trichocephalus* is much less affected by antihelminthics:—Hanau (1903) communicates a case where without doubt *Oxyuris* set up appendicitis: von Bégonin (1902) a case in which in an extirpated appendix he found the mucosa ulcerated and 15 *Oxyuris* in the lumen and Putnam (cited by Spieler (1904)) one in which 20 specimens were found: finally Schöppler (1906) holds that the danger of appendicitis is not removed when an *Oxyuris* which has wandered into the appendix dies; this is obvious if before death the worm has pierced the linings of the part and given exit to bacteria which may then exert a pathogenic action.

*Ascaris lumbricoides* (Linnaeus, 1758).

This, one of the commonest—as it is one of the largest—of the nematode human entozoa, normally inhabits the small intestine, but it is a little apt to wander and has in fact been found all over the body. It occurs at all ages, but is as a rule commoner in children about half

grown, and it is found in all climates, though it is more abundant in warm climates than elsewhere.

The genus *Ascaris* has, in certain of its species, the power of attaching itself to the inner lining of the wall of the alimentary tract. Guiart has described specimens of *A. conocephalus* from the intestine of a dolphin: "profondement incrusté dans la muqueuse, s'y était taillé une sorte de cupule assez profonde," and Weinberg has reported the case of an *Ascarid* lightly attached to the duodenal mucous layer of an ape and at this point there was ulceration. The latter writer quotes a letter from Dr Fontoynot, Professor at the School of Medicine, Tananarivo (Madagascar), in which he says that *A. lumbricoides* is of extreme frequency in the natives (Malgache), in whom, among other troubles, the worms frequently set up a mild appendicitis. He states "chaque fois que j'ai vu un indigène présenter du météorisme abdominal, de la péritonie légère, ou mieux du péritonisme avec localisation manifeste de la douleur au point de MacBurney, et épâtément dans la fosse iliaque droite, la santoline prise à la dose de 0.15 gr. a toujours fait évacuer un plus ou moins grand nombre d'ascarides et, par ce fait, a toujours amené la cessation de tous les phénomènes appendiculaires." The further fact that he states that with one exception he has not met with grave appendicitis amongst the natives, to some extent explains the immunity of the highly parasitized Chinese, an immunity which has led Martignon (1901) to doubt whether entozoa play any part in setting up appendicitis. Weinberg also recounts an observation made by Aldo Castellani on the extirpated appendix of a young girl, into which an *Ascaris* had penetrated and in which one half of its body was firmly fixed. Between the parasite and the walls of the appendix was a purulent fluid charged with *Bacillus coli*. Other cases of the impaction of the worm are recorded by Kelly and Hurdon (1905): by Bergmann (1890), in which case the *Ascaris* had bored through the walls of the appendix and attained the perivisceral cavity: Arboré-Rally (1900) regarded a severe case of appendicitis in a boy of 10 years as due to *Ascarids*: Triboulet (1901) regarded another case as due to *Ascariasis*: Schiller (1902) states that the disappearance of certain caecal abscesses after the expulsion of *Ascarids* supports the view that they were the cause of the disturbance and in this tends to confirm the views previously expressed by Czerny and Heddäus (1898); Schwankhaus (1901) found in the peritoneal cavity of a boy of 13, who had died of diffuse peritonitis, an *A. lumbricoides*, which had bored its way through from the appendix: Nason (1904) described a case in which an *Ascaris* inside the appendix had so coiled

itself and the appendix (like a finger in a glove) around the intestine as to cause an obstruction: Page (1906) records an operation on a man in whom appendicitis had been diagnosed, which revealed a number of *Ascaris* in the body cavity, and the specimens of this worm continued to make their way through the wound even eight days after the operation: other cases might be quoted, but I think enough has been said to show that in some cases *Ascaris lumbricoides* is an etiological cause of appendicitis and peritonitis.

*Trichocephalus trichiurus* (Linnaeus, 1771<sup>1</sup>).

Of all the common parasites in the human alimentary canal this is the one most generally recognized as causing appendicitis. Its normal habitat is the caecum and the colon, but it is found, though more rarely, in the vermiform appendix and in the small intestine. It occurs, with the exception of sucklings, in persons of all ages. It is cosmopolitan in its distribution, but is less common in the colder regions, though common in temperate climes. Braun (1908) states that dissection shows it to be present in the body in the following per cent. of those investigated in various places: Kiel 31·8%, Munich 9·3%, Göttingen 46·1%, Basle 23·7%, Greenwich 68%, Dublin 89%, Paris about 50%, and in Southern Italy almost 100% of the people are infected. Its presence as determined by the presence of the eggs in the faeces gives—where they are comparable—slightly different figures: Kiel 45·2%, Munich 8·26%, London 7·8% and Switzerland over 50%<sup>2</sup>.

Although the worm has been known, at least since the time of Linnaeus, but little has been done until recently to investigate its relations with the wall of the alimentary canal in which it lives. Askanazy (1896, p. 104) found that the *T. trichiurus* fed upon blood and that the only means of getting its food must be by piercing the mucosa. Wichmann (1889) made a painstaking examination of the subject, and his conclusions were that although the worm was so

<sup>1</sup> = *T. dispar* Rudolphi, 1801.

<sup>2</sup> These statistics date from some years ago and are probably not accurate for the present date. More recently French and Boycott (1905) found 7·8% of infection in 500 in-patients of Guy's Hospital, varying in age from a babe to over seventy. 84% of infections fell between the ages of five and forty, and of the patients examined between these years 11·75% were infected. I feel bound to add that in the opinion of these observers their research affords no support "to the notion that *Trichocephalus* has any aetiological relationship to appendicitis."



attached to the inner face of the intestinal wall that it required some slight force to withdraw it, this was due to its head-end being sunk in the mucus and coiled or wrapped round the villi. He found no evidence of lesions nor any solution of the continuity of the mucosa. Since Wichmann's time methods of research have improved and attention has been more closely focussed on the problem. Weinberg, whilst allowing that in many cases the whip-like fore-end of the body is simply hidden in the mucus, maintains also that "il y a des trichocéphales qui sont si bien fixés qu'en essayant de les détacher ou arrive plutôt à séparer le tronçon terminal de leur partie antérieure." In fact he maintains that the whip-worm is always fixed on the mucosa, and certainly some specimens we have at Cambridge confirm this statement. At times the anterior end passes through the mucosa and appearing again as a needle may be threaded through a curtain, at other times it hid its anterior end in a canal burrowed out in the mucous lining. Girard (1901, p. 265) has recorded finding two whip-worms in the extirpated appendix of a girl of eight, one worm had penetrated the mucosa and there was much inflammation about the lesion, numbers of mono- and polynuclear leucocytes and a copious bacterial flora were aggregated there. A similar inflammatory centre, surrounding the point of entrance of a whip-worm into the mucous layer of an idiot dead at the Vaulcluse Asylum, has been described by Vigouroux and Collet (1905, p. 270). Kaposi (1902) attributes a case of an appendicitis to the intervention of *T. trichiurus*. Moore (1906, p. 364) has recorded a case of appendicitis in which a "small worm was found"... "identified by Dr Thursfield as *Trichocephalus dispar*." Ovi (1906) found two specimens with their heads deeply embedded in the mucous layer in another appendix, and Kahane (1907) communicates the case of an appendix which on examination showed inflammation and in which were a number of specimens of *T. trichiurus*, some free and some embedded in the mucosa.

Amongst the most interesting cases are some recorded by Weinberg on the presence of nematode parasites in apes and monkeys. These animals are very subject to parasites and are very frequently infested with *T. trichiurus* as are also Lemurs. He gives a figure of the interior of the caecum of a *Macacus cynocephalus* which is riddled with scores of specimens of this worm. The monkey died after two days of fever and was at once examined, when all the organs were found congested. The caecum and colon contained hundreds of whip-worms, and histological investigation showed that the points of fixation of these worms were centres of inflammation which extended deeply into the wall of the

intestine and contained many polynuclear leucocytes and coli bacilli. The same microbes were obtained in cultures made from the blood. Weinberg considers the monkey died of an infection produced by a pathogenic organism introduced by the whip-worm into the walls of the intestine. He cites a further instance of a *Macacus sinicus* which died of septicaemia caused by *B. coli* and in whose intestine but one specimen of *T. trichiurus* was found, and he considers this one worm sufficed to bring about the fatal inoculation. The grave cases of anaemia described by many observers in cases of Trichocephaliasis may be due to similar inflammatory centres which as a rule are not visible to the naked eye but which are readily revealed on microscopic investigation.

Guiart and Grimbert (1906, p. 562) maintain that what they consider true of appendicitis (i.e. that the inflammation is set up by bacteria from the contents of the alimentary canal admitted to the tissues through punctures and perforations made by intestinal parasites) may also be true of Typhoid. They point out that if the bacillus of typhoid fever causes the disease entirely through its own efforts it is very difficult to understand why so small a percentage of people all drinking from the same water supply and all exposed to the same danger of wind or fly-borne infection suffer from the fever. If however the typhoid germs require a certain introduction to the walls of the alimentary canal one can understand its sporadic incidence and its association, formerly noticed, with the presence of *Ascaris* and *Trichocephalus* in the lumen of the intestine. An outbreak of typhoid occurred at Brest in the autumn of 1904. Investigating the dejecta of twelve patients suffering from the fever in the Hospital there Guiart found a constant passage of *Trichocephalus* eggs in ten of them. The number of eggs found in each case showed a strong infection. Of the two patients in whom there was not evidence that they were infected, one died and at the autopsy six specimens of *Trichocephalus* were found in his caecum. They may have been all males, or if females may have interrupted their oviposition; either alternative would explain the absence of eggs in the faeces. There was no opportunity of examining the caecum of the twelfth patient who apparently happily recovered, but his case may have resembled that of the man who died in whom the worms were found but whose dejecta showed no eggs. These numbers are too small to be conclusive and there seem to have been no control experiments, still they are at least suggestive. Guiart further states that renewed observation at Paris confirms the statement that *Trichocephalus* is

abundant in the intestines of typhoid patients except in children and that in them *Ascaris* seems to take its place as an inoculating agent.

The recognition of this association is no new thing. Roederer and Wagler in 1792 gave the earliest account of the "morbus mucosus" or typhoid fever, and they attributed the epidemic to the presence of the large number of intestinal worms (*Trichocephalus*) which on making autopsies were found in the alimentary tract. Pinel (1807) indicates that one should always suspect the presence of 'vers intestinaux' in cases of fevers of the mucous lining. Davaine has further noted the association of typhoid and worms, and other observers to the same effect are quoted by Guiart and Grimberty (1906).

An interesting confirmation of Guiart and Grimberty's views as to the part played by entozoa in typhoid fever is found in the following experiment of Weinberg (1906). Typhoid bacilli were given to two apes, one of which quickly died of septicaemia, the other survived repeated doses of the bacilli for 33 days, during which time its temperature rose at evening from 38.9° C. to 39.6° C. but there was nothing characteristic in the temperature chart. When the ape died (33rd day) the post mortem showed in the ileum a number of ulcerated Peyer's patches which presented the characteristic features of typical typhoid lesions in various stages of their evolution. The lower end of the duodenum and the upper end of the jejunum of this ape were full of a mass of tapeworms, some of which were found fixed at the level of the ulcerations. The caecum and the colon contained a great number of *Trichocephalus*. Examination of the blood and of the spleen by cultures demonstrated the presence of the typhoid bacillus and microscopic investigation of the ulcerations in the intestine confirmed the presence of the same germ in its walls; they also occurred in the small ulcerations which surrounded the point where the heads of the tapeworms were embedded. The authorities at the Pasteur Institute were satisfied that this was a true case of typhoid, and this is the more interesting as Grünbaum (1904) although he succeeded with an ape, failed to give a *Macacus* typhoid, though apparently Chantemesse and Ramond (1897) had succeeded previously. Soloukha more recently working under the same conditions, and with *B. typhosus*, failed to convey the disease to an ape. Weinberg concludes that success in his case was due to the fact that the burrowing into the mucosa of the taenia's head and suckers afforded a port of entry for the germs to the tissues, where they set up the ulcerations, and he states that there were masses of the

bacilli at the points where the suckers of the tapeworms were attached to the intestinal wall. Weinberg concludes this part of his thesis by saying that his microscopic sections show that:

(i) The tapeworm by fixing itself on the intestinal mucosa, sets up an intense congestion at the point of fixation.

(ii) At the same time it applies to this point of the intestinal mucosa such bacteria as are to be found on its suckers, and on the other hand it imprisons, between its suckers and the intestinal wall, such bacteria as existed before on this portion of the mucosa.

(iii) A considerable number of leucocytes make their way to the surface of the mucosa and take up the bacteria.

(iv) At other times, the bacteria penetrate into the thickness of the mucosa and set up inflammatory changes which may end in one of those ulcerations which are often found at the point of fixation of the tapeworm.

It seems then that Weinberg does not allow that the Cestode head breaks the continuity of the mucosa. He does not give precise details as to the species of "ténia" he is dealing with and it may very well be that the unarmed species do not penetrate the lining of the intestinal wall. But whoever will study Piana's Paper will I think have little doubt that in such genera of tapeworm as *Davainea*, and I think we may add *Hymenolepis*, there is a solution of the continuity of the lining mucosa of the host.

We must also not leave out of account the fact that some people and races are much more "tolerant" of all sorts of parasites, bacterial and others, and when infected suffer far less than do others who are susceptible to their action.

I am not quite sure how much injury to the mucosa is required to admit germs which are harmless within the gut lumen, but pathogenic when they gain free access to the blood or tissues, especially when the latter have been injured.

Without doubt the passage of the bacteria which set up intestinal disease is immensely aided by any agent which causes a lesion in the mucosa. Such lesions are normally caused in man—apart from any irritating substances he may swallow with his food, such for instance as the powdered diamond or glass which is said to have been used in Italy in the palmy days of poisoning—by entozoa.

I have in this paper confined my attention in the main to but three human intestinal parasites, all of them nematodes. There are, however,

many more which merit discussion, but these three are, from my point of view, the most important. Two of these, the *Oxyuris* and the *Trichocephalus*, are comparatively common, and the latter is probably much more common than is usually recognized. I have given some figures above as to its prevalence. The family Doctor knows how common *Oxyuris* is. Comparatively few children escape it and it attacks the rich and the poor, the apparently well cared for and the neglected, with complete indifference. Only a couple of months ago I found three specimens of *Oxyuris* in the extirpated appendix of a patient who was quite ignorant as were her parents that she harboured these worms. Further I have confined my attention largely to appendicitis, there are, however, many other diseases whose presence is associated with entozoa in the alimentary canal, e.g. certain forms of diarrhoea; some of these have been described by Weinberg who has investigated the relations of many more parasites to the intestinal wall than are considered here. All tell the same tale.

With the discovery of bacteria and the important work which has been done during the last forty or fifty years the grosser human parasites have been rather left in the shade. Before that time it was much more usual to administer vermifuges from time to time. Many of the numerous ailments of children were treated by our medical grandfathers with antihelminthics, and even to-day Sir Patrick Manson recommends that in the tropics and in other places where the intestinal parasites are common a course of santonin should be administered to children every six months. In spite of the great increase in our knowledge and practice of Hygiene, care in our meat supply, etc. which has so materially lessened the number of cases suffering for instance from the pork- or beef-tapeworm, I am not sure that, as regards other entozoa, whose entrance into the body is less easily controlled, we keep the inside of our digestive system as clean as our ancestors kept theirs. But times are changing, and increasing attention is being paid to what I am convinced is a serious factor in certain diseases. The matter is one which in England has received so far but little attention. Looking through the list of my "cloud of witnesses" hardly an Anglo-Saxon name occurs. Our knowledge of the relations of the parasite to the intestinal wall is derived mostly from Italian, French and German sources. In the United States however there is at least one voice crying in the wilderness. In Professor H. B. Ward's (1907) careful consideration of entozoa as germ carriers and germ inoculators, he says

"there has prevailed during recent years among the medical men of this country an exaggerated idea of the unimportance of human parasites. This must now give way to a proper conception of the pathological significance of these organisms, based upon careful investigations of their actual influence upon the host."

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## NOTE ON THE OCCURRENCE OF *TRIAENOPHORUS NODULOSUS* RUD. IN THE NORFOLK BROADS.

By A. E. SHIPLEY.

(1 Figure.)

A SHORT time ago Professor Minchin of the Lister Institute of Preventive Medicine sent me the liver of a Perch, *Perca fluviatilis* Rond. which was infested with cestode cysts. On examination each of these proved to contain a single specimen of the larval form of *Triaenophorus nodulosus* Rudolphi. The perch was caught in Sutton Broad, Norfolk, and, as I know of no record of this tapeworm being found in this locality, I have prepared this short note on its occurrence.

The cysts were spherical or oval, the diameter being about 1—2 mm. The cyst-wall was thick and laminated. Within each cyst a larval form of *T. nodulosus* (see Figure) was coiled or tangled up so as to accommodate its length (some 8—10 mm.) to its narrow home. Anteriorly the characteristic hooks and the two suckers are visible. There was no trace of segmentation in the body.



*Triaenophorus nodulosus* Rud. larva  $\times$  about 12. To the right one of the three-pronged hooks more highly magnified.



Zschokke<sup>1</sup> tells us that together with *Dibothriocephalus infundibuliformis* and the nematode *Cucullanus elegans*, *T. nodulosus* is one of the commonest of helminthes found in the fish of the Lake of Geneva. Like the first named of the above three worms it occurs in many species and in many individuals of those species. It further occurs all the year round. The cysts contain larvae in various stages of development. The cysts usually occur in the liver but they have also been found in the spleen, the muscles of the tail and in the walls of the intestine and in the body cavity, these last two situations harbour larvae in very early stages of development. They occur in some numbers, as many as thirty-six having been recorded from the same fish.

These larval forms attain at times a surprising length. Ordinarily from one to three centimetres long they have been found encysted in the tail muscles, eight, fifteen and even twenty-five centimetres in length.

These cysts have also been found in the tissues of the pike (*Esox lucius* L.) the normal host of the adult *T. nodulosus*, in the grayling (*Thymallus vulgaris* Nilss.), in the trout (*Trutta variabilis*) and in the salmon (*S. nuble*), but in the grayling, the trout and the salmon they attain very small proportions. Cysts have also been described from the pope fish (*Acerina cernua* L.).

The adult form normally inhabits the duodenum of the pike and lies with its head firmly fixed in the walls of the alimentary canal. It has also been found, though much more rarely, in the intestines of the fishes mentioned above.

<sup>1</sup> *Arch. Biol.* 1884, v. p. 153.

NOTE ON LEECHES SENT BY  
DR E. W. G. MASTERMAN FROM PALESTINE.

BY W. A. HARDING, B.A.

THESE specimens were preserved in alcohol and contained in three bottles *a*, *b* and *c*.

(*a*) contained 1 large and 47 small leeches "out of infested springs at Safed, Palestine."

(*b*) contained a leech "extracted from pharynx of an Arab of Jerusalem."

(*c*) contained a leech which had been removed, by means of laryngeal forceps, from the vocal cords of a young peasant in Jerusalem.

All these, as their history led one to expect, proved to be examples of *Limnatis nilotica* Savigny, 1820.

This leech has often been inaccurately described and confused with other species and in determining the specimens before me I have followed Professor Blanchard, who alone has given a satisfactory diagnosis.

The following description applies to Dr Masterman's leeches and agrees with Blanchard's account of *Limnatis nilotica*:

Number and arrangement of eyes and of intestinal caeca, and position of genital openings, as in *Hirudo*. Posterior sucker of large size. Upper lip of anterior sucker divided on its inferior surface into two lobes by a deep longitudinal groove. Jaws covered by papillae and provided with numerous sharp teeth. [N.B. Blanchard makes more than 100 teeth: I make less than 100.] Inhabits stagnant water, particularly drinking places, and invades the throat and nasal fossae of man and beast.

Blanchard gives the colour and size of *Limnatis nilotica* as follows:

Dorsal surface reddish-yellow or greenish, generally traversed by four black lines and occasionally by a median yellow or green strip. Two lateral orange stripes. Length 100—150 mm., breadth 10—15 mm.

The leeches from Palestine, being preserved in alcohol, cannot be expected to have retained their original colours. Traces of the lateral orange stripes appear in some of the small specimens in bottle (a).

The large specimen contained in (a) could extend itself during life to at least twice its present length, that is, to a length of more than 100 mm.; the two examples taken from the human throat are probably about half grown.

*Limnatis nilotica* has a wide distribution extending from the Azores, through Northern Africa and Egypt to part of Western Asia. It is a species to which the term "Horse-leech" has been applied and it has been often confused with the European Horse-leech, *Haemopsis* Savigny [= *Aulastoma* Moquin Tandon].

Savigny, whose figures are incorrect in certain respects, gives excellent drawings of this leech in a contracted and extended state and of the characteristic triangularly grooved lip referred to in my description of a leech from Angola [*Parasitology*, vol. I. p. 186].

A considerable literature exists relating to accidents caused by this leech, of a similar nature to those recorded by Dr Masterman.

Blanchard has collected much curious information on this point and gives many references.

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COMMUNICATION RECEIVED FROM THE SOCIETY FOR  
THE DESTRUCTION OF VERMIN.

95, WIGMORE STREET,

LONDON, W.

July 7th 1908.

TO THE EDITOR  
THE JOURNAL OF HYGIENE,  
FETTER LANE, E.C.

SIR,

*The War on Rats.*

In order to obtain accurate information regarding the nature and extent of the damage done by rats within the United Kingdom my Committee have prepared a schedule of questions which they desire to place into the hands of all those who are in a position, from their own experience, to give valuable information concerning temporary or permanent rat plagues in their districts, the damage inflicted by rats, the steps taken by them—individually or in co-operation with others—for preventing such damage, the means chosen for that purpose, and the results obtained.

As the only means of gathering important knowledge of that kind is by favour of the Press—short of undertaking the ‘appalling task of sending the questions to every one likely to suffer, or to have suffered, loss through rats, that is, to every householder in the country—my Committee venture to hope that you will permit an appeal to your readers to support the Society by asking for a copy of the schedule and returning it with such information as they may be able to impart.

I enclose a copy of the schedule for your information, and

am, Sir,

your obedient servant,

A. E. MOORE,

*Secretary.*

## (Schedule)

TELEGRAMS: "NIMREV, LONDON."

TELEPHONE NO. 48 PADDINGTON.

### THE INCORPORATED SOCIETY FOR THE DESTRUCTION OF VERMIN.

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95, WIGMORE STREET,  
LONDON, W.

Date.....

County of.....

Town (Village) of.....

.....Street

Number of Inhabitants.....

1. Name.....

(Profession, Trade, etc.).....

2. Description of place under report (state whether estate,  
farm, warehouse, factory, shop, etc.)

2A. Approximate area in square yards

2B. Number of persons living in the place

2C. Number of persons employed in the place

Total

2D. Describe the physical conditions of the district where  
your place is situated. Is there any factor favouring  
the existence of the rat plague?

3. How long has a rat plague existed, or how long have  
you been troubled with rats?

4. What are the particular kinds of loss inflicted by the rats?

- A. Destruction of Food  
 B. „ „ Material  
 C. „ „ Animal Life

- 4A. Can you approximately state in £ s. d. the loss caused in any one year?

in 190 about £

in 190 about £

in 190 about £

5. What means have you employed for destroying the rats (rat catchers, traps, bacteriological preparations, what kind)?

- 5A. What was the cost per year? About £

- 5B. Were the rats exterminated?

- 5C. How long did your place remain free from rats after using such measures?

6. In using a poison or a bacteriological preparation, have you suffered any loss through domestic animals dying from eating poison or virus, or eating rats killed with poison or virus? Please state full particulars.

7. Have you made any systematic efforts to deal with the rat trouble, either alone or in co-operation with others?

- 7A. If so, what were the means employed?

- 7B. What was the form of co-operation (state whether co-operation between neighbours, the residents of a parish, or several parishes, or of a rat and sparrow club)?

8. Can you state the approximate result of such systematic efforts?

Number of rats killed in 190

„ „ 190

„ „ 190

„ „ 190

„ „ 190

Remarks:

9. Can you mention any particular incidents illustrating the power of rats for doing injury?

*(Form A)*

THE INCORPORATED SOCIETY FOR THE DESTRUCTION OF VERMIN,  
LONDON.

GENERAL OBSERVATIONS.

1. In your experience, what is the best means on the market for destroying rats?  
(State what advantage it possesses in your opinion over other means).
2. Are you in favour of a proposal to multiply the number of the existing Rat and Sparrow Clubs, organising and co-ordinating them with similar agencies working for the extermination of rats?
3. If so, are you in favour of an annual grant being made by the State for that purpose, of say £10,000?

*(Form B)*

THE INCORPORATED SOCIETY FOR THE DESTRUCTION OF VERMIN,  
LONDON.

STATISTICS.

1. Is it in your opinion reasonable to assume (for the purpose of estimating the loss caused by rats, by the destruction of private and public wealth) that there is *at least* one rat to each acre of the total acreage of the United Kingdom?
2. Is it in your opinion reasonable to assume that the number of rats present on farms, in hamlets and villages is *at least* equal to the number of inhabitants?
3. Considering the greater facilities provided for rats in the towns, as regards food and hiding places, is it reasonable to assume that there are also in towns as many rats as there are human beings?
4. Is it in your opinion reasonable to estimate the economic loss caused by rats (through eating and spoiling food and destroying material) at one farthing per rat per day?

## SOME NOTES ON THE HAEMOGREGARINES PARASITIC IN SNAKES.

BY C. CLIFFORD DOBELL,

*Fellow of Trinity College, Cambridge; Balfour Student in the University.*

(From the Zoological Laboratory, Cambridge.)

(Plate XX.)

THE object of this paper is to describe the protozoan parasites which I have encountered in the red blood corpuscles of three different snakes—*Boa constrictor*, *Python spilotes* and *Coluber quatuorlineatus*. All the Protozoa are members of the genus *Haemogregarina*.

Before recording my observations, I wish to make some general remarks about the blood parasites of snakes and the work which has already been done on them. The literature of the subject is so scattered and so curiously muddled at present, that I think no excuse is necessary for my attempting to summarise our present knowledge. Although I fully agree with Minchin's remark that, "it is not new species of haemogregarines that are needed, but rather new facts about old species<sup>1</sup>," nevertheless, I think these notes may be not wholly useless. It is my hope that they may be of service to other investigators who are in a position more favourable for working out the life-history of these very interesting parasites.

In the first place, it must be pointed out that we are at present quite ignorant as regards the number of species of haemogregarines which have been found in snakes. It is by no means certain that different hosts always harbour different species of *Haemogregarina*. So long as this remains a matter of uncertainty, there is bound to be difficulty in naming the parasites. In our present state of ignorance, by far the most suitable nomenclature—it seems to me—is that which simply refers the parasite to its host. For instance, the parasite of *Python* would be called *Haemogregarina pythonis*. This method—even should the name subsequently prove to be a synonym—can lead to very little confusion, and is at present of considerable utility. Laveran and

<sup>1</sup> Minchin, *Proc. Zool. Soc.* 1907.



others have already adopted this system. The nomenclature which calls upon the observer's friends to supply the specific names of the parasites may lead to much confusion, and is heartily to be condemned [e.g. the haemogregarines of two species of python are called "*H. pococki*" and "*H. shattocki*" by Sambon]. And although the rules of zoological nomenclature are purely arbitrary, it is advisable—even for medical men—to adhere to them for the present.

Some confusion has arisen through the names of the snakes examined by various observers. For example, Langmann in 1899 described haemogregarines under "the generic name of haemosporidia" (*sic*) in *Spilotes couperi*. In 1901, Lutz described "*Drepanidium serpentium*" in *Coluber corais*. In *C. corais* Sambon (1907) also described a similar parasite, and without taking any notice of previous workers, named it "*Haemogregarina rarefaciens*." Now the snakes are all of the same species<sup>1</sup> in reality, and it is not improbable that the haemogregarines are also identical.

Another slight confusion in nomenclature has crept in through Börner (1901), who gave a table with a list of five haemogregarines and their hosts. Unfortunately, only one parasite is given to its proper host, the remaining four being distributed at random. This mistake has, apparently, been unwittingly copied by Minchin in his beautiful account of the Sporozoa (1903): so that three errors occur in his list of parasites and hosts.

At present nothing is known of *the way in which haemogregarines are transmitted* from snake to snake. Transmission may perhaps occur through the agency of an intermediate host (e.g. a tick)<sup>2</sup> or by way of the alimentary canal (as already described in similar frog parasites).

The *method of multiplication* appears to be by schizogony in the blood corpuscles—either whilst in the general circulation or in the viscera (spleen, lung, etc.).

No *sexual process* of any sort is known, and it is doubtful whether the forms described as males and females really are such. It will thus be seen that these animals afford a wide field for future work. It will, however, be needless for me to give further details regarding the various species. Instead, I have endeavoured to give as complete a bibliography as possible (p. 294) and have appended to my own observations a list of

<sup>1</sup> My authority for the names of the snakes has been throughout Boulenger's *Catalogue of Snakes in the British Museum*.

<sup>2</sup> Prowazek (1908) describes developmental stages (cysts etc.) in a pentastomid, *Porocephalus*. I regard these as exceedingly doubtful.

hosts and parasites (p. 292), which is as exhaustive as I have been able to make it. I may add that I have experienced considerable difficulty with the work of Sambon. As far as I am aware, Sambon has up to the present published merely a brief note in the *Lancet* (1907). Figures of his findings have been given, however, by Manson (1907), where many of the parasites are attributed to Sambon and Seligmann. Manson also gives some snake haemogregarines of which I can find no other mention. The information regarding them is, to say the least, scanty. In one place a new species is described from a "Mexican snake" under the name "*Haemogregarina brumpti* Sambon."

It may be remarked here that all the blood parasites of snakes—described under the names *Haemogregarina*, *Danilewskyia*, *Drepanidium*, etc.—probably belong to the genus *Haemogregarina*, though perhaps in part also to the genus *Karyolysus*. The genus *Haemogregarina* was created by Danilewsky in 1885—*Danilewskyia* Labbé 1894 being a synonym. If it be subsequently found that all the haemogregarines of snakes are really of the same species—which I think by no means impossible—then the correct name for the parasites is *Haemogregarina serpentium* Lutz.

#### I. *HAEMOGREGARINA* sp. from *Boa constrictor*.

(Plate XX, Figs. 1—13.)

This organism was obtained from a Brazilian *Boa constrictor* which had died of canker of the mouth. It is possibly identical with *Drepanidium serpentium* Lutz 1901 and *Haemogregarina terzii* Sambon 1907.

The parasites were very numerous, both in the general circulation and in the internal organs.

Small forms (Plate XX, Figs. 1, 2) were very uncommon. The commonest forms were those shown in Figs. 3—6. It will be seen that there are two distinct types of parasite—a long form with a recurved "tail" (Figs. 3, 4) and a stumpy form with rounded ends, and often with two vacuoles (Figs. 5, 6). The latter were not so frequently seen as the former.

Very large forms were fairly common, and many appeared to have arisen by the growth of the "tailed" forms (Figs. 7, 8). They attained a length of from  $13\mu$  to  $15\mu$ , and often appeared to have outgrown the corpuscles in which they had developed (Fig. 9).

In some of these large forms the nucleus had undergone partial fragmentation (Fig. 10), but I believe that this was the result of degeneration.

Certain of the parasites were distinguished by the possession of a very well-developed sheath (cytost), which stained a bright pink with Giemsa (Fig. 11). In other corpuscles I frequently found empty sheaths (Fig. 12), from which the parasites had evidently escaped. Free forms were also seen in the blood plasma (Fig. 13), and probably represent animals which have left their sheaths behind. The free forms attained a length of about  $17\mu$ .

I found doubly infected corpuscles on several occasions. No parasites were seen in the leucocytes.

From these facts it appears probable that the parasite enters a red blood corpuscle as a very small, falciform sporozoite or merozoite. In the corpuscle it grows into either a long or a stumpy organism—subsequently developing into a large fat form. It is possible that these two forms are sexually differentiated, and the large animals are possibly gametocytes, but this is merely conjectural.

At a certain period the parasite (? both forms) may envelope itself with a sheath (staining pink with Giemsa), from which it can subsequently issue. The significance of this is unknown.

I never found any forms which could be regarded as undergoing schizogony. Multiplication had probably ceased at this late stage of infection.

Details of structure will be readily seen by referring to the figures. It will be unnecessary for me to describe them more minutely here.

## II. *HAEMOGREGARINA* sp. from *Python spilotes*.

(Plate XX, Figs. 14, 15.)

I obtained this organism from an Australian *Python*. It is perhaps the same as *Danilewskyia pythonis* Billet 1895 (= *Haemogregarina pythonis* Labbé 1899) and *H. shattocki* Sambon 1907. Possibly *H. pococki* Sambon 1907 is another synonym. The haemogregarines of pythons have already been described by Billet 1895, Prowazek 1907, Sambon 1907 and Laveran 1908.

My organism is a typical haemogregarine (Figs. 14, 15) and shows no trace of the "blepharoplast" described by Prowazek. All the forms which I found were in approximately the same stage of development.

I have seen no differentiated "males" and "females" as described by Prowazek. Doubly infected corpuscles (Fig. 15) were several times seen.

Miss Robertson (1906) has described a haemogregarine-like organism from *Python* sp. under the name "*Trypanosoma pythonis*." It has a well-marked kinetonucleus, but I think it is premature to place it in the genus *Trypanosoma* at present. The animal does not appear to be the same as mine.

### III. *HAEMOGREGARINA* sp. from *Coluber quatuorlineatus*.

(Plate XX, Fig. 16.)

Haemogregarines have not previously been described from this snake, though they have been found in allied species. I have only been able to examine a single snake, and that was only very slightly infected. A blood smear (2 × 1 in.) showed usually but a single parasite after careful searching. All the animals seen were at the same stage in development, and presented the appearance of an ordinary haemogregarine (Fig. 16). There was never any indication of a blepharoplast.

A single *Coluber melanoleucus* was examined with negative results.

In conclusion, I wish to thank Mr W. A. Harding for his kindness in allowing me to examine the snakes in which the parasites herein described were found.

### LIST OF SNAKES INFECTED WITH HAEMOGREGARINES<sup>1</sup>.

(In alphabetical order.)

Snake	Haemogregarine
<i>Ancistrodon contortrix</i> ...	"Haemosporidia" (Langmann 1899).
<i>Ancistrodon piscivorus</i> ...	"Haemosporidia" (Langmann, 1899): <i>Haemogregarina mocassini</i> (Laveran 1902).
<i>Boa constrictor</i> ...	" <i>Drepanidium serpentium</i> " (Lutz 1901): " <i>Haemogregarina terzii</i> " (Sambon 1907): <i>Haemogregarina</i> sp. (Dobell).
[ <i>Bothrops</i> : see <i>Lachesis</i> .]	
<i>Bungarus candidus</i> ...	<i>Haemogregarina</i> sp. (Patton).
<i>Bungarus fasciatus</i> ...	" <i>Laverania</i> " <i>bungari</i> (Billet 1895) = <i>Haemogregarina bungari</i> (Labbé 1899).
[ <i>Coluber aesculapii</i> : see <i>C. longissimus</i> .]	
<i>Coluber corais</i> ...	"Haemosporidia" (Langmann 1899): " <i>Drepanidium serpentium</i> " (Lutz 1901): " <i>Haemogregarina rarefaciens</i> " (Sambon 1907).
(= <i>Spilotes couperi</i> )	

<sup>1</sup> The haemogregarines referred to Patton, are quoted from a personal communication from Capt. W. S. Patton, I.M.S., who is recording his observations elsewhere.

Snake	Haemogregarine
<i>Coluber longissimus</i> ... (= <i>C. aesculapii</i> )	<i>Haemogregarina colubri</i> (Börner 1901): "Haemogregarine" (Finkelstein 1907).
<i>Coluber quatuorlineatus</i> ...	<i>Haemogregarina</i> sp. (Dobell).
<i>Coluber</i> sp. ...	"Haemogregarine" (Simond 1901).
<i>Corallus cookii</i> ...	"Haemosporidia" (Langmann 1899): " <i>Haemogregarina lühel</i> " (Sambon 1907—according to Manson) <sup>1</sup> .
<i>Coronella getula</i> ... (= <i>Lampropeltis getulus</i> )	"Haemosporidia" (Langmann 1899): " <i>Haemogregarina wardi</i> " (Sambon 1907).
[ <i>Crotalus adamanteus</i> : see <i>C. scutulatus</i> .]	
<i>Crotalus confluentus</i> ...	"Haemosporidia" (Langmann 1899): <i>Haemogregarina crotali</i> (Laveran 1902).
<i>Crotalus scutulatus</i> ... (= <i>Crotalus adamanteus</i> )	"Haemosporidia" (Langmann 1899).
<i>Crotalus</i> sp. ...	" <i>Drepanidium serpentium</i> " (Lutz 1901) <sup>2</sup> .
<i>Dendrophis pictus</i> ...	<i>Haemogregarina</i> sp. (Patton).
<i>Drymobius bifossatus</i> ...	" <i>Drepanidium serpentium</i> " (Lutz 1901).
<i>Dryophis mycterizans</i> ...	<i>Haemogregarina</i> sp. (Patton).
<i>Elaps fulvius</i> ...	"Haemosporidia" (Langmann 1899).
<i>Eryz conicus</i> ...	" <i>Haemogregarina caniliei</i> " (Sambon 1907).
<i>Eryz johnii</i> ...	<i>Haemogregarina</i> sp. (Patton).
<i>Eunectes murinus</i> ...	" <i>Drepanidium serpentium</i> " (Lutz 1901).
[ <i>Eutaenia</i> : see <i>Tropidonotus</i> .]	
<i>Herpetodryas carinatus</i> ...	" <i>Drepanidium serpentium</i> " (Lutz 1901).
<i>Lachesis gramineus</i> ... (= <i>Bothrops viridis</i> )	"Haemogregarine" (Simond 1901).
<i>Lachesis mutus</i> ...	" <i>Haemogregarina seligmanni</i> " (Sambon 1907).
<i>Lachesis</i> sp. ... (= <i>Bothrops</i> sp.)	" <i>Drepanidium serpentium</i> " (Lutz 1901).
[ <i>Lampropeltis getulus</i> : see <i>Coronella getula</i> .]	
<i>Macroprotodon cucullatus</i> ...	" <i>Danilewskyia joannoni</i> ": " <i>Drepanidium</i> " sp. (Hagenmüller 1898).
[ <i>Morelia</i> : see <i>Python</i> .]	
<i>Naja tripudians</i> ...	<i>Haemogregarina najae</i> (Laveran 1902).
<i>Naja tripudians</i> var. <i>atra</i> ...	"Haemogregarine" (Simond 1901).
<i>Naja</i> sp. ...	"Haemogregarine" (Simond 1901).
<i>Philodryas olfersii</i> ...	" <i>Drepanidium serpentium</i> " (Lutz 1901).
<i>Psammophis sibilans</i> ...	" <i>Haemogregarina brendae</i> " (Sambon 1907).
<i>Pseudaspis cana</i> ...	" <i>Haemogregarina refringens</i> " (Sambon 1907).
<i>Python molurus</i> ...	" <i>Haemogregarina pococki</i> " (Sambon 1907).
<i>Python reticulatus</i> ...	" <i>Danilewskyia pythonis</i> " (Billet 1895) = <i>Haemogregarina pythonis</i> (Labbé 1899).
<i>Python spilotes</i> ... (= <i>Morelia spilotes</i> )	" <i>Haemogregarina shattocki</i> " (Sambon 1907): <i>Haemogregarina</i> (Laveran 1908): <i>Haemogregarina</i> sp. (Dobell).
<i>Python</i> sp. ...	" <i>Trypanosoma pythonis</i> " (Robertson 1906).
<i>Python</i> sp. ...	<i>Haemogregarina pythonis</i> Billet (Prowazek 1907).
<i>Rhadinaea merremii</i> ...	" <i>Drepanidium serpentium</i> " (Lutz 1901).
[ <i>Spilotes couperi</i> : see <i>Coluber corais</i> .]	

<sup>1</sup> The name is wrongly written "*Corellus cooki*."<sup>2</sup> By a misprint the snake is named "*Curotulus*."

Snake	Haemogregarine
<i>Spilotes pullatus</i> .. ...	" <i>Drepanidium serpentium</i> " (Lutz 1901).
<i>Tropidonotus fasciatus</i> ...	" <i>Haemosporidia</i> " (Langmann 1899).
<i>Tropidonotus ordinatus</i> ...	" <i>Haemosporidia</i> " (Langmann 1899).
(= <i>Eutaenia sirtalis</i> )	
<i>Tropidonotus piscator</i> ...	" <i>Haemogregarina mirabilis</i> " (Castellani and Willey 1904).
<i>Tropidonotus sauritus</i> ...	" <i>Haemosporidia</i> " (Langmann 1899).
(= <i>Eutaenia saurita</i> )	
<i>Tropidonotus stolatus</i> ...	" <i>Danilewskyia</i> " (Billet 1895) = <i>Haemogregarina</i> sp. (Labbé 1899).
<i>Tropidonotus viperinus</i> ...	<i>Haemogregarina viperini</i> <sup>1</sup> (Billet 1904).
<i>Vipera aspis</i> ...	" <i>Haemogregarina samboni</i> " (Giordano 1907—according to Manson).
<i>Vipera russellii</i> ...	<i>Haemogregarina</i> sp. (Patton).
<i>Xenodon newwiedii</i> ...	" <i>Drepanidium serpentium</i> " (Lutz 1901).
<i>Zamenis algirus</i> ...	<i>Haemogregarina algiri</i> (Manceaux 1908).
<i>Zamenis flagelliformis</i> ...	" <i>Haemogregarina mansonii</i> " (Sambon 1907).
<i>Zamenis hippocreptis</i> ...	<i>Haemogregarina zamenis</i> (Laveran 1902; Manceaux 1908).
<i>Zamenis mucosus</i> ...	<i>Haemogregarina</i> sp. (Patton).

To this list may be added the following :

"*Drepanidium*," found in "a puff-adder from Gambia" (Dutton, Todd and Tobey 1907).

"*Haemogregarina brumpti*" from a "Mexican snake" (Sambon 1907—*vide* Manson).

"*Haemogregarines*" from "several varieties of green snakes and the cobra found on the island of Hong Kong" (Hunter 1908).

According to Labbé, haemogregarines were first seen in snakes by Pfeiffer.

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<sup>1</sup> Emended (!) by Manson to *H. "viperina."*



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C. C. Dobell, del.





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## DESCRIPTION OF PLATE XX.

[All figures from permanent preparations stained by Giemsa's method. Drawings made under 8 mm. oil-imm. apochromatic obj. x comp. oc. 12 (Zeiss).]

Figs. 1-13. *Haemogregarina* sp. from *Boa constrictor*.

Figs. 1, 2. Young forms.

Figs. 3, 4. Larger forms, with slender recurved "tail."

Figs. 5, 6. Stumpy forms.

Figs. 7, 8, 9. Large forms.

Fig. 10. Large form with chromatin fragments in cytoplasm—probably degenerate.

Fig. 11. An organism lying inside its sheath (stained pink).

Fig. 12. Empty sheath in a corpuscle.

Fig. 13. Free form from blood plasma.

Figs. 14, 15. *Haemogregarina* sp. from *Python epiletos*.

Fig. 16. *Haemogregarina* sp. from *Coluber quatuorlineatus*.

## THE TRANSMISSION OF *TRYPANOSOMA LEWISI* BY FLEAS AND LICE<sup>1</sup>.

By GEORGE H. F. NUTTALL, F.R.S.

### (a) *Transmission by fleas.*

THE first experiments upon the transmission of *T. lewisi* by fleas were carried out by Rabinowitsch and Kempner (1899) who observed that three fresh rats placed with others harbouring trypanosomes in their blood subsequently (after 11—15 days) became infected. Fleas (species?) were found on these rats. They next teased the bodies of fleas taken from infected rats and inoculated them into fresh rats with the result that in five out of nine experiments the fresh rats became infected with *T. lewisi*. In one experiment they removed 20 fleas from infected rats and placed them on a clean animal with the result that the latter became infected after 2—3 weeks. They regarded this one experiment as conclusively proving that *T. lewisi* is transmitted by rat fleas.

Swingle (v. 1907, p. 119), who supposed he had observed a development of *T. lewisi* in rat fleas (species?), cites some indirect evidence which indicates that fleas play an important part in the transmission of the trypanosome. Of 17 one-fourth grown rats examined in the autumn and winter not one harboured lice, fleas or trypanosomes. Of seven rats examined in the spring, at the same place, all harboured fleas and four harboured trypanosomes. Rats captured in other parts of Lincoln, Nebraska, harboured neither fleas, lice, nor trypanosomes. Fleas were found more frequently than lice on trypanosome-infected rats.

The single experiment by Rabinowitsch and Kempner has hitherto constituted the sole proof that fleas transmit *T. lewisi*. I desire to record confirmatory experiments which were carried out with every precaution so as to exclude any other mode of infection.

<sup>1</sup> Read before the Cambridge Philosophical Society, 23 Nov. 1908. The third and fourth experiments with fleas have been added.

*Exp. I.* Three fleas (*Ceratophyllus fasciatus*) were removed from a wild rat (*Mus decumanus*) which was infected with *T. lewisi*. The fleas were immediately placed upon a white rat whose blood had been previously examined at intervals with negative results for about a month. The rat remained isolated. Daily examination of the rat's blood proved negative until the 7th day when *T. lewisi* was found.

*Exp. II.* Four days after the trypanosomes had first been found in the blood of the rat in Experiment I, the animal was killed and one flea was recovered. This flea was placed on another tame rat, but in this case no infection followed. (Experiments I and II were carried out in March, 1908.)

*Exp. III.* In this experiment<sup>1</sup> the tame rat upon which the fleas (*Otenophthalmus* [*Typhlopsylla*] *agyrtes* [Heller])<sup>2</sup> were placed was rigorously isolated in a flea-proof cage constructed after the pattern of those used by the Indian Plague Commission for their rat and flea experiments (see *Journ. of Hygiene*, 1906, Vol. VI. p. 435, Plate IV). The object of using this apparatus was to raise rat fleas in the laboratory. Eleven days (24. xi. to 4. xii. 1908) elapsed before trypanosomes appeared in the rat's blood :

Day 1. Blood examination of rat negative. Ten fleas were placed upon the rat, the fleas having been removed immediately before from a wild rat that was heavily infected with *T. lewisi*.

Day 3. Blood examination negative.

Day 4. Blood examination negative. Eight fleas were placed on the rat, the fleas having been removed immediately before from a wild rat which showed *T. lewisi* in its blood two days before it was killed and the fleas removed; no trypanosomes could be found microscopically on the day when the fleas were collected.

Days 5—10. Blood examination negative. On the 10th day, one flea from a wild rat infected with trypanosomes was transferred directly to the tame rat.

Day 11. Blood examination positive: 1 *T. lewisi* found in a fresh blood film.

<sup>1</sup> This experiment was carried out in conjunction with Messrs Patton and Strickland.

<sup>2</sup> Some of the fleas were kindly determined for me by the Hon. N. C. Rothschild, who states that *Otenophthalmus agyrtes* has not apparently been found before on *Mus decumanus*. All the rats harbouring *C. agyrtes* were captured in one spot (Cherryhinton Brook, Cambridge). It is possible that one or two *C. fasciatus* may have been amongst the fleas put on this rat, since out of a batch of 18 fleas collected from rats from this locality one specimen belonged to this species. In Exp. IV the living fleas were determined by me before being placed on the white rat.

Day 12. Blood examination positive : 3 *T. lewisi* found in a fresh blood film.

*Exp. IV.* Three fleas (*Ctenophthalmus agyrtes*) were removed from a heavily infected wild rat and immediately placed upon a clean white rat in a flea-proof cage. After 10 days (8—18. xii. 1908) many trypanosomes were detected in the white rat's blood.

In Experiment II a single flea failed to transmit *T. lewisi*. In Experiments I and IV three fleas transmitted the trypanosome. In Experiment III it is reasonable to suppose that the trypanosome infection was due to the first batch of ten fleas which was placed on the experimental animal. The ease with which infection took place through the agency of fleas suggests that they are probably the chief transmitters of the trypanosomes. An extensive series of observations which we are at present making will however determine the relative importance of fleas and lice in the transmission of *T. lewisi*.

(b) *Transmission by lice.*

In four experiments carried out by Rabinowitsch and Kempner (1899) lice (species?) were removed from rats harbouring *T. lewisi* in their blood. The lice were dissected and inoculated into clean rats, but the rats did not become infected.

MacNeal and Novy (vi. 1903, p. 560) observed living *T. lewisi* in the stomach of lice (species?), a statement which is repeated by MacNeal (xi. 1904, p. 520) who reports that in one experiment "several such lice" were transferred to a fresh rat (4. ii. 1904) with a positive result in that *T. lewisi* appeared in its blood after 14 days and persisted therein for five weeks. MacNeal was unable to observe any development of *T. lewisi* in the rat-lice although he frequently observed the trypanosomes in them. He concludes therefore that "the louse merely carries the protozoon from one animal to the other."

On the other hand Prowazek (1905, p. 365) described what he took to be the development of *T. lewisi* in the rat louse (*Haematopinus spinulosus* Burmeister), but he failed to transmit the trypanosome from rat to rat by means of the lice. Nevertheless he concluded that these lice are certainly capable of transmitting the trypanosome. He apparently based this statement solely on the supposed development of the trypanosome in the rat louse.

In this connection I have also a positive experiment to record: numerous lice (*H. spinulosus*) were removed from infected rats. Great care was taken to secure their not being injured. The lice were then transferred to clean white rats, being placed close to the skin and covered by smoothing back the rats' hair over them. The lice promptly attached themselves to the hairs of the new host.

*Exp. I.* 20. iii. 1908. Many lice were removed from a wild rat (*Mus decumanus*) in whose blood *T. lewisi* could not be found. The lice were transferred to a white rat (*A*) which had been infected by blood inoculation with *T. lewisi*, and showed trypanosomes in its blood on the day before the lice were applied.

24. iii. 30 lice were removed from rat *A* and placed on a clean white rat (*B*). Rat *A*'s blood showed many trypanosomes at the time when the lice were removed.

26. iii. 30 more lice were removed from rat *A* and placed on rat *B*. Rat *A*'s blood contained many trypanosomes.

3. iv. 1908. *T. lewisi* found in rat *B*'s blood for the first time.

A second experiment carried out with fewer lice gave a negative result.

*Exp. II.* 3. iii. 1908. Ten lice were removed from a wild rat (*A*) infected with *T. lewisi* and placed on a clean white rat *B*.

10. iii. Four lice were similarly removed from another infected wild rat (*A*) and placed on white rat *B*.

14—24. iii. 1908, and subsequently, rat *B*'s blood gave negative results on examination.

The first experiment with lice demonstrates that 30—60 lice are capable of transmitting *T. lewisi* from diseased to healthy rats. In the second experiment 14 lice failed to transmit the trypanosomes.

I desire to note that a large number of lice have been examined in the course of the last year with a view to studying the development of the trypanosome in the louse as described by Prowazek. I have been aided in this work by Mr C. Strickland. We have hitherto been quite unable to trace any development of *T. lewisi* in *H. spinulosus*, and we have begun to seriously doubt that such a development actually occurs. At present we incline to the opinion that Prowazek was deceived by the presence of extraneous flagellates such as are known to exist in a number of blood sucking arthropods. Such flagellates have proved a fruitful source of error of recent years and in consequence great caution is required before reaching any final conclusions regarding what may

perhaps appear to be developmental stages of trypanosomes in invertebrate hosts<sup>1</sup>.

Captain Patton, I.M.S., informs me that shortly after the publication of Prowazek's paper on the development of *T. lewisi* in *H. spinulosus*, he endeavoured to observe a similar development of *T. lewisi* in rat lice (*Haematopinus* sp.) in Madras. Beyond being able as we have done to recover the unchanged or degenerated trypanosomes he failed to find any of the stages described by Prowazek. Fleas (*Loemopsylla cheopis* Rothschild) also gave negative results on examination. Similar experiments with lice and fleas taken from the palm squirrel (*Funambulus palmarum*) infected with *Trypanosoma indicum* gave negative results.

In a preliminary note which has just been published by Manteufel (27. x. 1908) this author reports upon similar experiments. He states that he has been able to transmit *T. lewisi* by means of *H. spinulosus*, but gives no particulars regarding his investigations, reserving these for a paper which is to appear in the *Arb. u. d. Kaiserl. Gesundheitsamte*. He has in addition established the important fact that *H. spinulosus* is capable of transmitting *Spirochaeta recurrentis* from infected to healthy rats.

A series of 26 *Mus decumanus* which we carefully examined for *T. lewisi* and ectoparasites (30. x. to 3. xii. 1908) gave the following results:

				Ectoparasites present
3	harboured <i>T. lewisi</i>	...	...	Fleas only (15, 11, 1).
1	" "	...	...	Fleas (11) and lice (2).
1	" "	...	...	No fleas nor lice.
14	showed no trypanosomes	...	...	Lice on all of them.
1	" "	...	...	Flea (1) present.
6	" "	...	...	No ectoparasites.

The trypanosome rats all came from one locality, the uninfected rats came from three other localities. These observations again demonstrate the local character of the infection and support the view that fleas are the usual agents concerned in the transmission of the trypanosome. In a future paper we shall deal more fully with this aspect of the problem.

*The experiments above recorded clearly demonstrate that T. lewisi is transmitted from rat to rat by means of Ceratophyllus fasciatus, Ctenophthalmus agyrtes and Haematopinus spinulosus.*

<sup>1</sup> This aspect of the problem is discussed in another paper by Patton and Strickland in this number of *Parasitology*.

*Since three distinct kinds of blood-sucking insects are capable of transmitting T. lewisi it appears doubtful that this flagellate is a parasite of the invertebrate "host" in the sense claimed by Prowazek and other investigators.*

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ON THE PRESENCE OF AN ANTICOAGULIN IN THE  
SALIVARY GLANDS AND INTESTINES OF *ARGAS*  
*PERSICUS*.

By GEORGE H. F. NUTTALL, F.R.S.  
AND CYRIL STRICKLAND, B.A.

IN the literature relating to the Ixodoidea there are a number of cases recorded of injurious effects following the bites of ticks. We do not refer to diseases like piroplasmosis and spirochaetosis which are known to be tick-transmitted, nor to other infective processes which may start at the seat of the tick's bite. The effects we refer to follow almost immediately upon the infliction of the bite and are distinctly toxic in character. These effects appear to have been more frequently observed following upon bites inflicted by species of *Argas* and *Ornithodoros*<sup>1</sup>.

From the fact that strangers to a district are apt to suffer more severely than do the natives, it has been concluded that repeated tick-bites bring about a condition of immunity similar to that which has been observed in the case of mosquito bites. This appears to support the view that ticks give off something of the nature of a poison when inflicting their bites. On the other hand the toxic effects are by no means constant. In fact, in the case for instance of *A. reflexus*, the bites are only occasionally followed by immediate ill effects, and from this it has been argued that the persons who suffer possess a peculiar susceptibility or idiosyncrasy with regard to the poison.

It has not as yet been suggested that the differences in the after effects of the bite may also be due to differences in the substance injected into the wound by the tick. That this suggestion may however require consideration is indicated by an observation one of us has made with regard to a specimen of *O. coriaceus* (♀). The tick in question was captured in a wild part of Mexico, far removed from any human habitations. It is problematical from what source

<sup>1</sup> The literature on the subject has been fully dealt with elsewhere by one of us (1899, 1908), and need not be further considered here.



the tick had derived its food before it attacked the collector. The tick was removed almost immediately after it attached itself and it is doubtful if it actually drew any blood before it was forcibly detached. The bite produced a large violet ecchymosis within a few minutes and it took months for the wound to heal. On arrival in Cambridge this tick was allowed to bite a fowl with the result that a large haemorrhagic spot appeared about the wound, whilst the tick was feeding, and within half-an-hour or so an irregularly circular ecchymosis had formed measuring about two inches across. After the meal of fowl's blood had been digested it was again fed on a fowl on two occasions, but in neither case did any reaction take place around the seat of the puncture.

When fowls or other animals have been bitten by *A. persicus* or *O. moubata* in Cambridge, in the great majority of cases no reactions occurred about the minute punctures, but occasionally small ecchymotic areas appeared pointing, as in the case of *O. coriaceus* above cited, to a direct toxic action.

It is not known if ticks do or do not regurgitate material from their digestive tract whilst feeding. Should it be proved that regurgitation occurs, then an explanation of the occasional toxic effects might be found in the character of the regurgitated material which has been derived from a previous meal. On the other hand our experiments show that there may be considerable differences in the amount of anticoagulin which is present in the salivary glands of individual ticks. We may conclude from this that the amount of effective secretion injected into the wound may vary. But leaving hypothesis aside, it appeared desirable to learn something by experimental methods regarding the possible character of the salivary gland secretion.

A careful search of the literature has only brought to light one paper bearing upon the subject from the experimental standpoint. The investigations of Sabbatani (1898—1899), at Cagliari, demonstrated that the bodies of *Ixodes ricinus* (♂ and ♀) contain a substance which retards or prevents coagulation,—an anticoagulin. We have been able to go a step further by demonstrating the presence of anticoagulin in the salivary glands and intestines of *A. persicus*.

Sabbatani carried out his experiments as follows:

He removed replete female ticks from dogs, cut them in pieces with scissors, rubbed them up in a mortar together with salt solution, and filtered the fluid through muslin. Having added a known quantity of salt solution to a definite weight of ticks he utilized quantities of "tick solution" which corresponded to a given number of ticks

(i.e., 1 c.c. of solution corresponded to 1 tick). He found that 3—4 c.c. of solution prevented the coagulation of 20 c.c. of human and dog blood for 24 hours, that 8 c.c. of solution prevented the coagulation of 25 c.c. of ox and sheep blood, that 1 c.c. of solution prevented the coagulation of 5 c.c. of guinea-pig blood. When only 2 c.c. and 6 c.c. of solution were added to 25 c.c. of ox and sheep blood respectively, coagulation was markedly retarded. The anticoagulin also acted upon pig and frog blood.

When the solution was injected intravenously into dogs and their blood was sampled after intervals of 3—25—40 minutes, it was found that the blood samples did not coagulate or coagulated very slightly after 24 hours. The dose administered corresponded to about 1 gramme of tick per kilo of dog. The effect was less evident in the case of the cat, rabbit and guinea-pig. Lymph taken from the thoracic duct of dogs treated with the "tick solution" did not coagulate. A solution made from *male* ticks likewise exerted an anticoagulating action *in vivo*.

On heating the solution to boiling (100° C.), for 5—10 minutes, it no longer exerted an anticoagulating action. He extracted the active principle in the manner that Haycraft (1884)<sup>1</sup> did for "*hirudin*" in the case of leeches. He added absolute alcohol to the solution, collected the precipitate, dried it and redissolved it in salt solution. The extract prepared in this manner also contained anticoagulin.

Sabbatani found that intravenous injections of the solution produced grave effects in all the animals upon which he experimented: rapid and marked decrease in blood pressure; rapid heart-beat, soon followed by stoppage of the heart's action; respiration was slowed and then stopped. If the animals did not die whilst the injection was being practiced, they showed profound prostration, loss of reflexes, even complete paralysis. Moderate doses, administered to dogs and cats, caused diarrhoea, vomiting, loss of coordination, tremor, decreased blood pressure and rapid pulse, and the animals, after remaining feeble for hours, slowly recovered. Small doses exerted little or no effect. Dogs were very susceptible to the action of the tick extract, cats less so, whilst cattle and sheep were relatively resistant.

Blood corpuscles exposed to the action of tick extracts did not become hæmolyzed but they became crenated after 8—12 hours. The leucocytes appeared to be more resistant than the corpuscles.

The observations of Sabbatani have been quoted thus at length for

<sup>1</sup> *Arch. f. exper. Pathol. u. Pharmacol.* XVIII. p. 209.

the reason, already stated, that they represent the only scientific experiments bearing on the subject<sup>1</sup>. It will be noted that he extracted the anticoagulin from the tick as a whole and made no attempt to determine in which organs of the tick the antibody was present.

#### *Methods.*

The experiments here detailed were made with *A. persicus* obtained from South Africa through the courtesy of Mr C. P. Lounsbury, Government Entomologist, Cape Colony.

The salivary glands of the ticks were isolated by dissection under the microscope, they were rinsed off in .8% salt solution to remove extraneous impurities, and were then crushed between two clean slides in a minimal quantity of fluid. By raising the edge of one of the slides, the fluid gathered near one edge and practically the whole amount was then allowed to flow into a capillary tube. The tube was calibrated so as to measure about .02 c.c. of fluid. When the gland emulsion had flowed in up to the mark, it was followed up by an equal quantity of blood taken as it flowed from a needle prick on the experimenter's finger. The gland emulsion and blood were then mixed up by alternately blowing out the fluid into a clean watchglass and then drawing it up into the capillary. The mixture was then drawn up into a clean capillary and allowed to stand for varying periods before it was examined. The examination consisted in blowing out the capillaries at stated periods to determine if coagulation had taken place or not. Control tubes were prepared in which an equal volume of salt solution and blood were mixed; in every case coagulation took place within a few minutes (7—8') in the controls. Several preliminary experiments showed that salivary gland emulsions exerted a marked anticoagulating action. We record the following:

#### *Experiments with Salivary Gland Emulsion and human blood.*

*Experiment I.* One gland of *A. persicus* (♀, 6 mm. long) was emulsified in .02 c.c. of salt solution and mixed with .02 c.c. of blood. Result: Coagulation delayed for 30 minutes.

*Experiment II.* The glands of 4 *A. persicus* (♀, average length 7 mm.) were emulsified in .05 c.c. of salt solution. The emulsion was

<sup>1</sup> We have been unable to consult the paper by Mosso (1899), but believe it contains only a report on Sabbatani's experiments. Grützner (1902) published a note with a suggestive title, but no contents either worth noting or original.

used concentrated and diluted to various degrees so that  $\frac{1}{2}$  to  $\frac{1}{16}$  of active principle was contained in the .02 c.c. of saline which was mixed with an equal volume of blood. Result :

Dilution 1	prevented clotting completely.
" $\frac{1}{2}$	delayed clotting for 40 to 120 minutes.
" $\frac{1}{4}$	" " " 30 minutes.
" $\frac{1}{8}$	" " " 20 "
" $\frac{1}{16}$	" " " 15 "

*Experiment III.* The glands of 4 *A. persicus* ( $\delta$ , average length 6.5 mm.) were emulsified in .05 c.c. of salt solution, the emulsion being used concentrated and diluted as in Experiment II. Result: Dilutions 1,  $\frac{1}{2}$ ,  $\frac{1}{4}$  all prevented clotting completely.

*Experiment IV.* The glands of 10 *A. persicus* ( $\delta$ , 5.5 to 7.2 mm. long) were emulsified separately in .02 c.c. of salt solution and each mixed with equal volumes of blood:

The glands of Tick No.	Delayed coagulation for	Size of Tick (length) in mm.
1	90 minutes	7.2
2	45 "	7
3	45 "	6.5
4	prevented completely	6.2
5	45 minutes	6
6	75 "	6
7	75 "	6
8	75 "	6
9	95 "	5.7
10	75 "	5.5

*Experiment V.* This experiment was made to determine if the emulsion of tick salivary gland exerted any deleterious effect on living leucocytes maintained at 35° C. The emulsion was mixed with a minute drop of human blood and the leucocytes were observed under the microscope.

(a) One gland was emulsified and mixed with blood.

*Effect:* Nil. Leucocytes actively motile after 2 hours.

(b) One gland in strong emulsion was mixed with blood.

*Effect:* Nil.

In none of the foregoing experiments was there any haemolysis observed even when gland emulsion was added in large amount to the blood.

*Experiment with Rabbit's blood.*

*Experiment VI.* A similar result was obtained with rabbit blood. The blood contained in the control tube clotted in 8 minutes, that in the tube containing salivary gland emulsion only clotted after 75 minutes.

From these experiments we conclude that the salivary glands of *A. persicus* contain a substance which prevents coagulation of the blood. The stronger the emulsion of gland the greater is the anticoagulant action. The amount of anticoagulin in the glands of different ticks varies considerably (Experiment II) and bears no relation to the size of ticks measuring 5.5 to 7.2 mm. in length. The amount of anticoagulin present in the glands will doubtless be found to depend upon the state of functional activity of the organs. The amount of anticoagulin present in the glands of a single tick is sufficient to prevent the coagulation of .02 c.c. of human blood for 45 to 95 minutes, or even longer. The salivary gland emulsion exerts no inhibitory effect on the movements of leucocytes in extravascular blood and neither does it exert any haemolytic effect upon the red blood corpuscles.

*Experiments made with emulsions of the intestines and human blood.*

Having established the fact that anticoagulin is present in the salivary glands of *A. persicus*, it remained to be determined if anticoagulin is present in the intestines.

*Experiment VII.* The intestines of 3 *A. persicus* were dissected out, rinsed in salt solution, emulsified in a small drop of salt solution which was mixed in the proportion of 1:4 with blood.

Clotting was delayed for 2 hours. The corpuscles were not haemolysed. It is evident from this experiment that anticoagulin is present in the tick's intestines, and that haemolysin is absent.

*Experiments in vivo.*

It having been stated by Alt (1892, cited by Nuttall, 1899, p. 46) that an emulsion prepared by crushing 3 *Argas reflexus* produced toxic effects in a dog (comparable to those produced by small amounts of snake venom) on subcutaneous injection, it appeared desirable to control this observation. An emulsion of the body contents of 6

*A. persicus* was, therefore, prepared and injected subcutaneously into a mouse. The injection produced no noticeable effect. Salivary gland emulsion was similarly injected into a mouse (glands of 6 *Argas*), a cock (glands of 5 *Argas*) and a rabbit (glands of 5 *Argas*), but in no instance was the slightest local or general effect produced.

*The effect of temperature upon the anticoagulin in the salivary glands of A. persicus.*

In the case of the specific anticoagulin for rabbit's blood<sup>1</sup> Bordet and Gengou (1901) found that the antibody resists heating to 58·5° C. Sabbatani states that the anticoagulin present in *Ixodes ricinus* is destroyed by an exposure to 100° C. for 5—10 minutes.

To determine the temperature at which the anticoagulin in *A. persicus* is rendered inactive, we proceeded as follows: The glands of several ticks were dissected out and emulsified in salt solution, after which the emulsion was drawn up into capillary tubes. Some of the tubes were set aside as controls, others were heated to various temperatures for a period of 10 minutes. The capillaries which were heated were attached by elastic bands to a thermometer which was kept moving to and fro in a waterbath maintained at the temperature desired. After having been heated the emulsion was mixed with blood and tested in the usual way.

In a preliminary experiment, made with the glands of one tick, the unheated emulsion prevented coagulation for over 4½ hours, whereas an emulsion heated to 55° C. was to some extent inactivated since complete coagulation of the blood with which it was mixed took place after 90 minutes.

In the next experiment an emulsion of the glands of several ticks was used. In the tube containing unheated emulsion coagulation commenced to take place after 61 minutes, whereas the heated tubes gave the following results:—

Heated to	Complete coagulation after
65°	23 minutes
70°	16 "
75°	12 "
80°	8 "

<sup>1</sup> Produced by injecting rabbit's blood into guinea-pigs. See Nuttall (1904), *Blood Immunity and Blood Relationship*, p. 17.

It follows that the anticoagulin is destroyed by an exposure for 10 minutes to a temperature of 80° C. and that its activity is reduced by lower temperatures, being considerably reduced even by a temperature of 55° C.

#### CONCLUSIONS.

1. There is clinical and experimental evidence that the bites of *Argasidae* may be occasionally followed by toxic effects which are either local or general in character.

2. This toxic effect may be due either to the peculiar susceptibility of the individual upon whom the bite has been inflicted or to the character of the substances injected into the wound by the tick in the act of biting. The cause of the toxic effect remains to be discovered.

3. The bodies of *Ixodes ricinus* contain substances which prevent the coagulation of the blood and cause toxic symptoms when injected into dogs. These substances do not cause haemolysis (Sabbatani).

4. The salivary glands and intestines of *A. persicus* contain anticoagulin but no haemolysin.

5. The amount of anticoagulin present in the salivary glands of *A. persicus* varies considerably. The amount contained in the glands of a single tick may delay the coagulation of .02 c.c. of human blood for 45 to 95 minutes or indefinitely. The anticoagulin also acts on rabbit's blood.

6. The movements of human leucocytes remain unaffected by exposure to emulsions of the salivary glands of *A. persicus*.

7. Excepting the effects due to the presence of anticoagulins, it has not been established that the bodies or salivary glands of *A. persicus* contain toxic substances.

8. The anticoagulin in the salivary glands of *A. persicus* is destroyed by an exposure of 10 minutes to a temperature of 80° C. Its action is partially abolished by a similar exposure to 55° C.

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#### *Note whilst going through the press.*

We have omitted to mention an interesting observation by Christophers (1906, p. 45) with regard to the fluid excreted from the "coxal glands" of *Ornithodoros savignyi*. Christophers casually remarks that "the fluid is slightly alkaline and prevents the coagulation of the

blood." He gives no further particulars. Colonel W. B. Leishman informs us that he has observed the same phenomenon with regard to the fluid excreted by *O. moubata*. He was led to suspect that the fluid might exert an anticoagulating effect because he observed that drops of blood, escaping from punctures made by *moubata*, failed to coagulate when they mixed with the fluid discharged by the ticks upon the skin of the host. Nuttall (1908, pp. 83, 97, 102) has observed the excretion of a similar fluid by *O. coriaceus* and *Argas persicus*.

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INOCULATION OF DOGS WITH THE PARASITE OF KALA  
AZAR (*HERPETOMONAS* [LEISHMANIA] *DONOVANI*)  
WITH SOME REMARKS ON THE GENUS *HERPETO-  
MONAS*.

BY CAPTAIN W. S. PATTON, I.M.S.

SINCE the discovery of the parasite of Kala Azar by Lt.-Col. Leishman, R.A.M.C., a number of investigators have endeavoured to reproduce the disease in the lower animals but without success. The recent discovery of Nicolle that dogs are susceptible to an allied human parasite (*Herpetomonas infantum*) has added a fresh stimulus to work along these lines, and in order to see whether the dog can be infected with *H. donovani* I carried out some inoculation experiments, which I propose recording in this paper.

Three young dogs were selected from a number of specimens brought to the King Institute; they had no ticks on them nor were they infected with *Piroplasma canis* and beyond being somewhat emaciated they were good specimens of bazaar dogs. Through the kindness of Lt.-Col. Robertson, I.M.S., I was able to obtain 10 c.c. of splenic blood from two typical cases of Kala Azar, and on examining some films it was found to be rich in parasites.

The dogs were inoculated as follows :

	Date	Weight	
Dog A.	9 June, 1908.	550 grms.	Inoculated with 2 c.c. intra-hepatic and 2 c.c. intra-peritoneal.
Dog B.	9 June, 1908.	306 grms.	Inoculated with 2 c.c. intra-hepatic and 2 c.c. intra-peritoneal.
Dog C.	9 June, 1908.	1124 grms.	Inoculated with 2 c.c. intra-peritoneal.

The inoculations were carried out with the greatest care, so that there could be no doubt that all the dogs received large numbers of the living parasites. The dogs were kept in clean iron cages free from ticks, their blood being examined at intervals with negative results. On June 19th the dogs were weighed and found to be as follows: Dog A, 820 grams, Dog B, 392 grams and Dog C, 1246 grams. On

June 30th Col. Robertson punctured the spleen of another case of Kala Azar and I obtained 6 c.c. of blood rich in parasites; Dogs *A* and *B* were each inoculated intra-peritoneally with 3 c.c. of this blood.

Dog *B* was killed on August 4th, when it weighed 514 grams. The following are the post mortem findings:

*Spleen.* Weight 12½ grams, it appeared quite healthy; eight smears were made from different parts of the pulp and stained with Giemsa's stain. I was unable to find any parasites in them.

*Liver.* Weight 118 grams, there were no macroscopic appearances to be noted; eight smears were made from different situations, and were stained with Giemsa's stain. No parasites could be found.

The kidneys, lungs, intestines and peritoneum were quite healthy, and no parasites could be found in the bone marrow.

Dog *A* was killed on August 11th, when it weighed 1272 grams.

*Spleen.* Weight 26 grams, no macroscopic changes; 12 smears were made as above, but no parasites were found.

*Liver.* Weight 255 grams, healthy; eight smears were made, but they contained no parasites.

All the other organs were healthy.

Dog *C* is at present in the Institute at Madras.

From these experiments it would therefore appear that the dog is not susceptible to *Herpetomonas donovani*. The few dogs I have had the opportunity of examining in Madras have never harboured this parasite, and Christophers, who has had a much larger experience with these animals, does not record its occurrence.

Should it eventually prove to be the case that no animals are susceptible to the parasite of Kala Azar, it would further support the view that the Indian and Assam species is distinct from the Tunisian organism. I have been unable to collect any evidence in Madras that would support the view that dogs play any part in the transmission of Kala Azar. In Madras it is essentially a house disease and few of the people in Georgetown, where it is very prevalent, keep dogs.

### *The Genus Herpetomonas*<sup>1</sup>.

Believing as I do that the parasite of Kala Azar and its two allies belong to the genus *Herpetomonas*, I have for the last two years made a special study of this genus. These flagellates are parasitic in the

<sup>1</sup> This portion of the paper should be read in conjunction with my description of *Herpetomonas lygaei* in the *Archiv für Protistenkunde*, Vol. XIII. p. 1, 1908.

intestinal tracts of insects, non-blood-sucking and blood-sucking, and it should be clearly understood that those species occurring in the latter have no connection with any blood parasite, nor are they cultural trypanosomes; they have a characteristic developmental cycle which may be conveniently divided into three stages, *pre-flagellate*, *flagellate* and *post-flagellate*.

In their pre-flagellate stages they are round or oval bodies of varying size, and contain two characteristic chromatic bodies, the nucleus and blepharoplast. In this stage they multiply by simple longitudinal fission or by multiple segmentation, and are found in the midguts of their insect hosts, except in the case of the three human parasites. I have been able to show that this stage in a known species occurring in *Culex* mosquitoes is exactly similar to that of the human parasites. The flagellate stage is characterised by the formation of a *single* flagellum and the multiplication of the resulting flagellates by equal or unequal longitudinal division. The adult forms are long, spindle-shaped organisms, with a single flagellum, but no undulating membrane. This stage occurs in the mid- and hindgut of their hosts, but in the case of *Herpetomonas donovani* it takes place naturally in *Cimex rotundatus*. The post-flagellate stage is characterised by the massing together of the flagellates in the midgut, their shortening and rounding up, the resulting cysts are passed out in the faeces and are accidentally sucked up by fresh hosts. It is not known yet whether *H. donovani* undergoes this stage in the bed-bug.

It is important to remember that many of these *Herpetomonads* are indistinguishable in their *pre-flagellate stages*, and therefore if this stage alone is studied two distinct species may be classed as one. Further, I wish to point out here that a partial study of the stages of these flagellates is very apt to lead to confusion, so that what are true *Herpetomonads* may quite easily be mistaken for *Crithidia* or young *Trypanosomes*; a reference to recent literature will show that this has actually occurred in more than one instance.

As the life-cycles and general structure of the three human parasites are similar to those of well-known *Herpetomonads*, I see no reason for placing them in a distinct genus. The differences in their development, such as the formation of the flagellum, methods of division and the fact that their *pre-flagellate* stages are passed in man only justify their being regarded as specifically distinct from such species as *H. muscae domesticae*, *H. sarcophagae*, *H. culicis*, *H. lygaei*, and many others.

## A TRYPANOSOME AND HAEMOGREGARINE OF A TROPICAL AMERICAN SNAKE.

By C. M. WENYON, M.B., B.S., B.Sc.

*Protozoologist to the London School of Tropical Medicine.*

### Plate XXI.

THE trypanosome to be described in this paper was discovered in blood films taken from the snake *Erythrolamprus aesculapii* (Duméril and Bibron) of tropical America. For these films I am indebted to Dr Leiper. In addition to the trypanosome there was present in the blood a haemogregarine. Though haemogregarines are very common in snakes, especially in the Tropics, where nearly every snake examined is found to harbour these parasites, the reverse is the case with trypanosomes. Several observers have recorded the presence of trypanosomes in snakes but hitherto no one has given an accurate description of one of these either in the living or stained condition. Indeed our knowledge of the trypanosomes of the whole group of reptiles is very limited when compared with other groups of Vertebrata. On this account it seems of interest to place on record the characters of this trypanosome as it appears in the blood films mentioned above.

In the forthcoming third volume of *Reports of the Wellcome Research Laboratories*, Khartoum, I have described under the name of *Trypanosoma najae* a trypanosome of the spitting cobra *Naja nigricollis*. The trypanosome of the cobra was only met with in wet films and in spite of prolonged examination of many stained films not a single example of the stained trypanosome could be found.

In the present instance only three stained films (Leishman stain) of the blood of *Erythrolamprus aesculapii* were available and the appearances of the trypanosomes in these films are as follows.

Two main types can be recognised and as with many other trypanosomes these may be distinguished as the wide and narrow forms. The

total length of both these varies from 30 to 35  $\mu$ . The width of the narrow forms is from a half to a third of that of the wide forms. Both are characterised by having the non-flagellar end much drawn out beyond the micronucleus or nucleus as the case may be. It may be so marked as to give the appearance of a whip-like process or flagellum.

The micronucleus is usually rod-shaped and prominent and is very uniform as regards its position in the body of the parasite. With reference to the nucleus its position varies but this is produced by the change in position of the nucleus rather than by any alteration in the situation of the micronucleus.

The flagellum arises from a point near the micronucleus and is never directly connected with this latter structure. It pursues a slightly wavy course along the border of the undulating membrane to the extremity of the body whence it is prolonged as a free flagellum for a distance of 5.5 to 7  $\mu$ . The flagellum is a delicate structure which does not stain very deeply and is developed to about the same extent as in *Trypanosoma lewisi*.

The nucleus consists of a roughly spherical group of fine chromatin granules and varies considerably in its position in the body of the parasite. It may be at the middle of the body (Fig. 4), or close to the micronucleus which, as pointed out above, varies very little in position. When in the latter situation, the nucleus may be on the flagellar side of the micronucleus as is usual in trypanosomes (Figs. 1 and 3), or it may be on the non-flagellar side (Fig. 5), as in the genus *Herpetomonas*. The occurrence of both forms in the blood of any animal is not common. *Herpetomonas* forms have been noted in the case of *Trypanosoma lewisi* and in *Trypanosoma theileri*. More recently such a condition has been described in *Trypanosoma wrublewskii*. In invertebrate hosts it is more common and Minchin has recently shown that a herpetomonas form occurs in the life cycle of the trypanosome of *Glossina palpalis*.

The flagellar extremity of the trypanosome is blunter than the very pointed non-flagellar end.

The protoplasm of the parasite stains blue and is free from granules. It shows a marked alveolar structure, while in the narrow forms it is as a rule darker than in the wider forms. Such a condition might lead one to suspect that the wide forms are merely flattened out narrow trypanosomes. From an examination of a large number of trypanosomes it is clear that this explanation is improbable. To some observers the wide and narrow forms would appear as female and male respectively but nothing is known of the history of this trypanosome and nothing in

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support of this can be deduced from an examination of the stained blood films.

The body of the parasite is usually curved with the undulating membrane running on the convexity. This curve of the body probably represents the natural condition, for such a disposition of the body, though more marked, was a characteristic feature of *Trypanosoma najas* of the cobra.

Measurements of two of the trypanosomes were as follows:

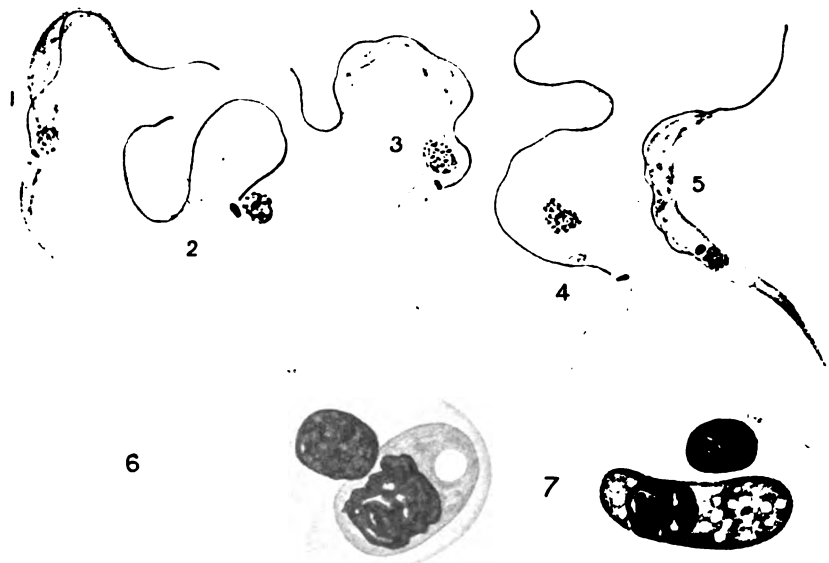
	Form with nucleus and micronucleus separate	Form with nucleus and micronucleus adjacent
Non-flagellar extremity to micronucleus	9.8 $\mu$ }	9.8 $\mu$
Micronucleus to nucleus	4.9 $\mu$ }	
Length of nucleus	2.1 $\mu$	2.1 $\mu$
Nucleus to flagellar extremity	11.2 $\mu$	11.2 $\mu$
Free flagellum	5.6 $\mu$	7.0 $\mu$
Total length of body	33.6 $\mu$	30.1 $\mu$
Width of body	4.2 $\mu$	2.8 $\mu$

The trypanosomes in the blood were fairly numerous: about one parasite to every 20 or 30 fields was the rule.

For this trypanosome which cannot be identified with any known species I propose the name *Trypanosoma erythrolampri* from its host *Erythrolamprus aesculapii*.

#### *Haemogregarina* sp.

It has been mentioned that a haemogregarine occurred with the trypanosome just described. This parasite differs very little from some of the haemogregarines already noted from other snakes. The red corpuscles are slightly increased in size and their nuclei displaced by the presence of the parasite. In other respects there is no recognisable change. The parasite usually occurs as an elongated slightly curved body with rounded ends measuring about 12.5 by 5  $\mu$  (Fig. 7). There is a delicate membrane or cyst enclosing the parasite which has its narrower end turned up. The cyst is less marked than it is in many haemogregarines. The protoplasm is vacuolated and the nucleus large and deeply staining. Some of the parasites are shorter and broader (Fig. 6) than the type just described but they show the same vacuolated protoplasm and deeply staining nucleus. The infection was a small one, there being many more trypanosomes in the films than haemogregarines.







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## DESCRIPTION OF PLATE XXI.

*Trypanosoma erythrolampri* Wenyon, and *Haemogregarina* of *Erythrolamprus aesculapii* (Duméril and Bibron).

- Fig. 1. Narrow trypanosome with nucleus adjacent to micronucleus.  
 Fig. 2. Wide trypanosome with nucleus at side of micronucleus.  
 Figs. 3, 4. Two wide forms with true trypanosome structure.  
 Fig. 5. Narrow form with nucleus and micronucleus as in *Herpetomonas*.  
 Figs. 6, 7. Two haemogregarines showing short and long form.

## THE HAEMOGREGARINES OF MAMMALS AND REPTILES.

BY CAPTAIN W. S. PATTON, M.B. EDIN., I.M.S.

(*From the Quick Laboratory, Cambridge.*)

IN two recent papers, Sambon and Seligmann (1907, 1908) have recorded some observations on the intracellular parasites of snakes, and have described no less than ten new species. The authors, in discussing the life histories of these parasites, have made the startling discovery that, "the life history of the *haemogregarines* like that of the *haemoprotozoa* is divided into two cycles: a schizogonic or 'vegetative' cycle spent in the blood of vertebrates and characterised by asexual multiplication, and a sporogonic or sexual cycle spent in the digestive organs of blood-sucking invertebrates and characterised by sexual reproduction." The authors then go on to speak quite familiarly of young *merozoites*, adult *schizonts*, adult *sporonts*, and so on.

My excuse for making some remarks on their findings is the fact that since November, 1905, up till July, 1908, I have worked with the following snakes infected with haemogregarines:

<i>Bungarus coeruleus (candidus)</i>	<i>Eryx johnii</i>
<i>Vipera russellii</i>	<i>Gonglyophis conicus</i>
<i>Naja tripudians</i>	<i>Dryophis mycterizans</i>
<i>Python molurus</i>	<i>Dendrophys pictus</i>
<i>Zamenis mucosus</i>	<i>Tropidonotus piscator</i>
	<i>Tropidonotus stolatus</i>

In all 250 snakes were at one time or another in the Laboratory at the King Institute, Madras, the majority of which harboured two species of *Aponomma*: *A. gervaisi* and another species not yet identified. Careful feeding experiments with the larvae, nymphs and adults of both these ticks were carried out in special receptacles, so that the conditions were very much as they occur in nature. In addition, I have had the

opportunity of studying no less than five haemogregarines in *Rana tigrina* and *Rana hexydactyla*, not only in the frogs but in the leech which transmits them. I have also studied the haemogregarine of *Emyda granosa* both in the tortoise and in the transmitting leech.

Lastly, I have had the unique opportunity of studying three mammalian *Leucocytozoa*: *L. funambuli*, *L. felis domestici* and *L. leporis*. With this large material at my disposal I have made an exhaustive effort to trace out the extracorporeal life histories of these intracellular parasites of mammals and reptiles, but in every case I have failed to find any developmental cycle in the corresponding blood-sucking invertebrates. In the case of the leech from *Emyda granosa*, Christophers once showed me some bodies which suggested developmental forms of the haemogregarine of the tortoise; I have examined these parasites in the leech, but can only come to the conclusion that they probably represent some stage in the life cycle of a *Coccidium* parasitic in the leech.

A few observations on *L. leporis* in the tick *Haemaphysalis flava* have suggested to me that the method of transmission of these parasites will eventually prove to be mechanical and that the characteristic vermicules,—*whose sex, by the way, I am at present unable to determine*,—free themselves in the intestinal tracts of the various invertebrate hosts and in some manner at present unknown make their way back to the biting parts. I have actual experimental evidence proving that the vermicules of *L. leporis* can remain alive in the alimentary tract of larvae and nymphs of *H. flava* for at least 15 days.

I am at a loss, therefore, to understand how Sambon and Seligmann have been able to observe adult *sporonts*, *schizonts*, etc., and how they are in a position to state these parasites have a *sporogonic* cycle in invertebrate hosts. It remains to be seen what observations they have made on the curious cycle found in the lungs and liver of snakes infected with haemogregarines. I have examined many examples of all the stages of this cycle not only in the stained condition but particularly in the fresh condition (for 12 hours) in the lung of *Zamenis mucosus*, and I have not been able to satisfy myself as to whether this cycle of multiplication is an asexual or sexual process; for this reason I could not definitely say what the different forms seen in the peripheral blood of all my snakes really represented. Yet I find Sambon and Seligmann call some of these *merozoites*, others adult *schizonts*, *sporonts*, etc. As far as I can gather they have principally studied these parasites in the peripheral blood of snakes, so that I cannot see their grounds

for these statements. Further, I would point out that I have examined haemogregarines in eight different genera of snakes and from a study of the parasites not only in the peripheral blood but also in the organs, I believe they belong to the same species. On the contrary Sambon and Seligmann make every haemogregarine they see in different species of snakes' peripheral blood a new species even in spite of the fact that they studied the parasites in this country when probably not a single snake had a tick on it. Without infecting a snake through the agency of the right tick and then studying the various forms of the parasites that appear in the blood and the organs of the snake, I do not see how it is possible to speak of the parasites in the peripheral blood as *schizonts*, *sporonts*, etc.

During the 2½ years I have studied these intracellular parasites I have not felt myself justified in recording the results of my observations, as I considered my work would in no way advance our knowledge of these parasites, but after reading Sambon and Seligmann's papers I feel it is right I should record them, even though they are negative. Many keen observers in the tropics just beginning the study of these parasites and with excellent material at hand, on reading Sambon and Seligmann's papers, may come to the conclusion that there is nothing new to be learnt about them. I would like to advise them that this is not the case and that in my opinion the work of Sambon and Seligmann, instead of adding to our knowledge of these parasites, has increased the confusion already existing.

NOTE. While the above article was in the press a paper by Prowazek came to my notice. Prowazek speaks of free vermicules and cysts having a membrane with a double contour. The parasites he describes occurred in the Pentastome from a Python infected with *Haemogregarina pythonis*. He suggests that the cysts represent a further development of the haemogregarine. In November 1905, I examined a large number of Pentastomes (*Porocephalus pattoni*, Stephens) which are very common in the lungs of the rat snake *Zamenis mucosus* and found they were infected with what I then thought represented developmental forms of the haemogregarine of the snake. In addition to many free vermicules the Pentastomes contained cysts with a membrane having a double contour and containing smaller cysts full of spindle shaped bodies. I now know these cysts represent part of the cycle of a parasite peculiar to the Pentastome and have nothing to do with the haemogregarine of the snake.

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A CRITICAL REVIEW OF THE RELATION OF BLOOD-SUCKING INVERTEBRATES TO THE LIFE CYCLES OF THE TRYPANOSOMES OF VERTEBRATES, WITH A NOTE ON THE OCCURRENCE OF A SPECIES OF *CRITHIDIA*, *C. CTENOPHTHALMI*, IN THE ALIMENTARY TRACT OF *CTENOPHTHALMUS AGYRTE*, HELLER.

BY CAPTAIN W. S. PATTON, M.B. EDIN., I.M.S.,  
AND C. STRICKLAND, B.A.

(*From the Quick Laboratory, Cambridge.*)

IT is now an established fact that a number of blood-sucking arthropods and leeches are infected with flagellate organisms belonging to the genera *Herpetomonas* and *Crithidia*, and we believe that in more than one instance, these natural flagellates have been described as developmental forms of various trypanosomes which might be ingested by these sanguivora. As the whole question of the transmission of trypanosomes is intimately connected with these so-called developmental forms in invertebrate hosts, we propose reviewing in detail this important subject before recording our observations on the flagellate of *Ctenophthalmus agyrtes*.

It will be remembered that the late Dr Schaudinn (1904) was the first to give a detailed description of the life-cycle of a trypanosome (*T. noctuae*) in a blood-sucking invertebrate, *Culex pipiens*. Although he referred to the similarity between his developmental forms in the mosquito and *Crithidia fasciculata* of Léger (1902), he made no reference to the possibility of his mosquitoes being infected with similar flagellates. Novy (1907) and his collaborators have clearly shown that both *Herpetomonas* and *Crithidia* may occur in the same mosquito, and one of us (1907) has traced the development of *Herpetomonas culicis* Novy from the larva of the mosquito through the nymph up to the adult insect, showing that even when mosquitoes are bred in the laboratory from larvae caught at large they may be infected with this flagellate.

Without actually repeating Schaudinn's experiments at Rovigno it is quite impossible to unravel his elaborate paper and to say whether he was dealing with a *Herpetomonas* or a *Crithidia* as well, not to mention the possible presence of *Spirochaeta culicis* in his mosquitoes<sup>1</sup>.

Following Schaudinn's work, Prowazek (1904) claimed to have discovered the development of *T. lewisi* in the rat louse, *Haematopinus spinulosus*; but no subsequent observers, so far as we know, have found these developmental forms in rat lice. We have dissected and examined a large number of *Haematopinus spinulosus* from rats well infected with *T. lewisi*, but beyond finding unchanged<sup>2</sup> and degenerating trypanosomes we have never seen any of the forms described by Prowazek. One of us has also endeavoured to trace out the development of *T. lewisi* in rat lice, *Haematopinus* sp.?, in Madras with negative results. We are therefore forced to the conclusion that Prowazek has described part of the life-cycle of a natural flagellate, *Crithidia*, of *Haematopinus spinulosus*, and as such we believe it has no connection with *Trypanosoma lewisi*. We have examined Prowazek's figures and have no hesitation in saying they are exactly similar to appearances seen in other insects infected with *Crithidia*. For instance his figure 53 at once recalls an agglomerated mass of *Crithidia* such as one of us (1908) has recently depicted in the case of *Crithidia gerridis*; his figure 44 we would regard as a typical adult *Crithidia*, and his fig. 42, as a young form showing the development of the flagellum. It is important to note that Prowazek was unable to infect rats with lice which presumably had these developmental forms in them, nor does he mention the occurrence or not of these *Crithidia* in lice from rats uninfected with *T. lewisi*.

It is therefore of the utmost importance that this work of Prowazek should be confirmed or otherwise by those who have the opportunity of searching for this *Crithidia* of *Haematopinus spinulosus* in lice off rats from the same localities Prowazek obtained his 40 rats, viz. Berlin, Trieste and St Pelagio near Rovigno. We have searched so far in vain for this parasite in England. It is quite possible it is localised in its distribution.

The next important work on the development of Trypanosomes is that of Koch (1905), Gray and the late Captain Tulloch (1905). These

<sup>1</sup> In this connection see also Ross (1906, pp. 96, 101), Nuttall (1906, p. 109), Novy, MacNeal and Torrey (1906, p. 110).

<sup>2</sup> By this term we mean we have never seen parasites exhibiting changes such as occur in the *pre-flagellate* stages of *Herpetomonas* or *Crithidia*. We have certainly seen thin active trypanosomes which appear to have resulted from longitudinal division of ordinary forms.

observers stated they had discovered the development of *T. gambiense* and *T. brucei* in the *Glossinae*, but the later work of Novy (1906), Minchin, Gray and Tulloch (1906) has shown that tse-tse flies harbour natural flagellates in their alimentary tracts, and that these parasites are not connected with the pathogenic trypanosomes, *T. gambiense* and *T. brucei*. Minchin (1908), in his recent studies of these flagellates, made the important discovery that one of them, *T. grayi*, encysts in the rectum of *G. palpalis*; although at first agreeing with Novy that it is not connected with a vertebrate trypanosome, Minchin now regards it as an avian trypanosome. Minchin however does not attempt to explain how the cysts of *T. grayi* find their way back to the fly; it is presumed they are ingested by some bird, pass into its blood, and are again taken up by the fly when feeding on the bird. This extraordinary hypothesis appears to be based solely on the habits of the tse-tse fly as it is known to feed exclusively on the blood of vertebrates. In our opinion this fact does not exclude the possibility of these flies accidentally ingesting the cysts passed out in the faeces of other flies. At present very little is known of the habits of the *Glossinae*, and in order to understand how they may ingest these cysts it is important to find out whether they congregate at their breeding grounds, what they do shortly after hatching out, whether they fly off immediately to get a feed of blood or whether they remain sometime at their breeding grounds. These are certainly the occasions when they may accidentally ingest cysts passed out by other flies on leaves, twigs, etc. It is well known that blood-sucking species of Tabanids insert their proboscides into dew and other fluids on leaves, etc. when running about on these objects at their breeding grounds. It is important to examine tse-tse flies before they have had their first feed or even immediately after to see whether they contain any stages of *T. grayi* or other flagellates. In any case, we believe the cysts of *T. grayi* and their development into flagellates should be searched for in the alimentary tract of *G. palpalis*. The fact that Minchin (1908) and Stuhlmann (1907) found bacteria in the stomachs of tse-tse flies further confirms our view that these flies may become infected with other organisms, and we can find no proof either in Minchin's paper or in Stuhlmann's that these bacteria are derived from the blood these flies ingest or are inherited by them.

The single instance of a fly bred in captivity becoming infected with *T. grayi* after feeding on a fowl Minchin would regard as proof positive (*sic*) that this flagellate is an avian parasite. We can, however, find no mention of the fowl having trypanosomes in its blood, and Minchin



appears to have overlooked the possibility of the fly having parasites in its stomach before it fed on the fowl. Further, we find that this particular fly was bred from a pupa in August 1905, and that it was kept in a fly cage in which presumably other wild flies had been kept, so that it is not impossible for the fly to have ingested cysts of *T. grayi* passed out in the cage in the faeces of other flies, and as it was not killed till October 10th, 1905, there was ample time for this to take place and for the parasites to germinate; we think this is in the highest degree probable. It is fruitless to discuss this point any further; far more exact experiments and proofs are required before it can be accepted that *T. grayi* is an avian trypanosome.

Koch (1905) was the first to study the development of pathogenic trypanosomes in the *Glossinae*, *G. palpalis*. He states (1907) that he observed the development of *T. gambiense* in *Glossina fusca* and *Glossina tachinoides*. He differentiates the parasites into short and slender forms, zygotes with many nuclei which give rise to small forms. All Koch's attempts to inoculate animals with these forms have however proved negative. It is really difficult to see what connection these so-called developmental forms have with *T. gambiense*. Koch now regards the crocodile as a source of blood supply for tse-tse flies, and, in consequence of his statements it has been gratuitously assumed, more especially by the lay press, that the flies obtain the pathogenic trypanosomes from crocodiles. This assumption is entirely unjustified.

Turning now to Stuhlmann's (1907) recent work we find this observer dealt principally with *G. fusca* bred from pupae; the flies being fed for the first time on calves, sheep and dogs infected with *T. brucei*. After 2 to 4 days, 80—90% of these flies developed a rich infection of flagellates in their alimentary tracts. Stuhlmann describes long forms which are found in the proventriculus and oesophagus but rarely in the proboscis; small forms which are almost exclusively found in the proboscis and seldom in the gut. He also describes amoeboid forms with or without flagella and regards them as resting parasites. Having studied these various forms Stuhlmann summarizes the development of *T. brucei* in *G. fusca* as follows: the cycle begins by a multiplication of indifferent forms in the intestine of the fly, the parasites then spread forward to the proventriculus where conjugation takes place, and as a result of this process small forms are produced which Stuhlmann believes are destined to pass into the vertebrate host. He was unable to find parasites in the end gut or any of the other organs. As a result of these observations on *G. fusca* bred from pupae and subsequently fed

on infected blood (*T. brucei*), Stuhlmann considers the above cycle represents the development of *Trypanosoma brucei*. It is, however, important to note that he was unable to infect animals with any of the forms he describes. Before criticising Stuhlmann's work it will be necessary to refer to two flagellates one of us has recently studied in Madras.

When endeavouring to follow the vermicules of *Leucocytozoon leporis* in the tick, *Haemaphysalis flava*, from *Lepus nigricollis*, the pre-flagellate stage of a flagellate was found in the gut diverticula of the larval tick; these forms corresponded exactly with similar stages of *Crithidia gertrudis*, *Herpetomonas donovani* and *H. lygaei*. In fed larvae, kept for a few days, further development of these forms was observed, viz. the development of the flagellum, multiplication and formation of adult *Crithidia* and in the nymphs these flagellates were found in abundance. It was also discovered that only a certain percentage of the larvae from one adult was infected. Naturally the question immediately arose where had these flagellates come from? At first the only reply was, from the blood of the hare. Forty-two of these animals were examined when studying *L. leporis* and the blood of two on which a very large number of larvae was fed were frequently examined for months, both in the fresh and in the stained conditions, and, although many of the larvae had flagellates, no such parasites were ever seen in the blood of the hares. We are aware that a trypanosome (*T. cuniculi*) has been found by Jolyet and de Nabias (1891), Nicolle, Petrie (1904), Bosc (1904), and Bettencourt and França (1906), in *Lepus cuniculus*, and also that it is quite an easy matter to miss these parasites in the peripheral blood of animals. Yet, in spite of these facts, we believe that the flagellate found in the tick is in no way connected with a vertebrate trypanosome; the final proof of this will be dealt with elsewhere. Many hundreds of recently fed larvae of *Haemaphysalis flava* were examined, but we never found a trypanosome in any of them. As a result of these observations it was concluded that this *Crithidia* of *H. flava* is transmitted hereditarily. Swingle has informed one of us that he has worked out the mode of infection in the case of *Crithidia melophagia*; he finds that it is also transmitted hereditarily, thus confirming our observations on the hereditary transmission of *Crithidia*.

The other flagellate referred to above is found in the crop diverticula of a species of *Glossiphonia* sp.? parasitic on *Rana tigrina* in Madras. In addition to a number of haemogregarines, this frog is infected with two species of trypanosomes. After a long series of feeding experiments

with young leeches it was found that in one particular batch from one parent a large percentage developed an intense flagellate infection, while of another batch of young leeches fed on the same infected frog not a single one developed these flagellates, although exactly the same species of leech was used. It was eventually found that the explanation was quite simple: if the parent leech had flagellates in its alimentary tract a large percentage of its young also had them, whereas if the parent was not infected, its young also never developed flagellates. It was further impossible to trace any connection between these flagellates and the frog trypanosomes; we therefore believe they are true leech flagellates which are transmitted hereditarily. It is interesting to note that the flagellates developed in the leech from 2 to 4 days after feeding, exactly as in the case of the flagellates of *G. fusca*, and the small round forms of the leech flagellate were nearly always found in the anterior diverticula of the crop of the leech (cf. findings of Stuhlmann in *G. fusca*). So that in spite of the fact that Stuhlmann examined the other organs, presumably also the ovaries of *G. fusca*, we believe that the flagellate of this fly is transmitted hereditarily. We know it is exceedingly difficult to demonstrate the parasites in the eggs so that unless they were specially searched for at a particular stage they may readily be missed. In support of our view we would point out that Stuhlmann never found encysting forms similar to those of *T. grayi* in the rectum of *G. fusca*. Further Stuhlmann makes no reference to control experiments, that is to say feeding flies for the first time on animals known to be quite clean; we also note that no mention is made of the examination of the alimentary tracts of pupae of *G. fusca* descended from flies infected with flagellates. On examining Stuhlmann's figures (Plate X, figs. 151 to 158) we are unable to see what connection the flagellates represented have with *Trypanosoma brucei*. Figures 152 *a* to *g* are described as trypanosomes from a heavily infected fly; of these *a* is a good picture of a young dividing *Crithidia*, *b* another form showing the growth of the flagellum; the remaining figures *c* to *g* represent elongated *Crithidia* undergoing division; it is interesting to compare these figures with those of *Crithidia gerridis*. The proof as to how *T. brucei* comes to develop into these forms is in our opinion entirely wanting in Stuhlmann's work.

In a recent paper Keysseltz and Mayer (1908) claim to have fully confirmed Stuhlmann's observations on the development of *T. brucei* in *Glossina fusca*. These authors at the outset refer to Prowazek's work on the development of *T. lewisi* in *Haematopinus spinulosus*; we have

referred fully to this work above and have clearly shown that there is no evidence to support Prowazek's view of the flagellates he found in the rat louse. Keysselitz and Mayer, being unable to get sufficient material to breed out flies, *G. fusca*, studied the development of *T. brucei* in freshly caught insects. Judging from the contents of their intestines it would appear that all the flies had fed solely on mammalian blood, as no nucleated red blood corpuscles were seen. It seems to us to be very dangerous to presume that these caught flies had fed on animals infected with *T. brucei* alone, but this is apparently what Keysselitz and Mayer have done. 4.6% of these flies were, according to them, infected with *T. brucei*, whereas 11.2% which had been fed on healthy animals after being caught, were similarly infected; this difference is explained by saying that the parasites enter a swarming period and multiply after a meal when they are found between the "Epithel und Darmwand." The authors found the parasites in the proventriculus, proboscis, fore- and midgut, and in all cases in which they were in the proboscis they were also found in the other situations. All the flagellates we are told had the same general character, so that Keysselitz and Mayer regard them as representing part of the cycle of *T. brucei* as proved by Stuhlmann. We consider this proof is wanting for the reasons we have mentioned above.

In one hungry fly Keysselitz and Mayer saw many amoeboid non-flagellate forms as well as motile parasites between the "epithelium and intestinal wall." In the juice of the proboscis they found agglutinated stages of small trypanosomes which were attached in the proboscis on the oral side of the openings of the salivary glands; they conclude that they might be washed into the wound when the salivary glands empty themselves. The authors, however, do not say whether they tried to inoculate animals with these forms.

Keysselitz and Mayer fed their freshly caught flies on cattle which had become spontaneously infected with *T. brucei*; the blood of the cattle, they say, contained male and female forms of *T. brucei*, as described by Prowazek (1905). We are not convinced of the certainty of Prowazek's male, female and indifferent forms of *T. brucei*, and we have not seen any process of conjugation in this parasite. Even if it is admitted that there may be sexual dimorphism, Keysselitz and Mayer do not tell us how the zygote develops into the parasites they speak of in *G. fusca*. It is not at all clear to us why Keysselitz and Mayer fed their flies on infected animals, for they state that the flies had fed at large on animals and were infected with *T. brucei*. In these flies further development was

not seen, in spite of the fact, as the authors tell us, that they had fed on blood containing male and female forms of *T. brucei*. In another series of 96 freshly caught flies fed on the cattle, 10.4% became subsequently infected with *T. brucei*, and it will be observed that this percentage corresponds to that of freshly caught flies, though, if they were feeding on suitable material containing male and female forms of *T. brucei*, it is only natural to expect more would become infected. We would not however have expected this, as we regard these flagellates of *G. fusca* as natural parasites. Keysselitz and Mayer also found that 11.2% of freshly caught flies subsequently fed on healthy rabbits became infected, they do not explain how this took place. As their results are in direct opposition to Stuhlmann's observations on flies (*G. fusca*) bred from pupae when 80—90% became infected, Keysselitz and Mayer explain this discrepancy by the fact that only 10% of Stuhlmann's flies subsequently retained the infection. In our opinion the fact that in the majority of the flies the parasites tend to disappear from the alimentary tract, the longer the flies are kept, clearly suggests that the parasites have passed out of the digestive tube in their migration to the ovaries.

Keysselitz and Mayer conclude that *G. fusca* is only capable of being infected once in the course of its life, that is to say it is only once capable of offering conditions suitable for the further development of *T. brucei*, and that is when it takes its first feed of blood (from special animals) which contain male and female forms of *T. brucei*. All the trypanosomes which may be subsequently ingested die out, and this explains why other observers have failed to study the further development of *T. brucei* in freshly caught *Glossina fusca*. Why subsequently ingested male and female trypanosomes do not develop we must confess we do not understand.

Keysselitz and Mayer examined the "Geschlechtsproducte"<sup>1</sup> of four female *fusca*, and it would thus appear they at least suspect the possibility of the flagellates of the fly being transmitted hereditarily. Throughout Keysselitz and Mayer's paper we can find no reference to the possibility of these flagellates of *G. fusca* being anything more than harmless parasites of the fly and which have no connection with *T. brucei*. Although it is clear the only possible way they could be transmitted from fly to fly is by hereditary infection,—no encysted stages being found,—neither Stuhlmann nor Keysselitz and Mayer has carried out exhaustive feeding experiments to disprove this.

<sup>1</sup> By "Geschlechtsproducte," literally "sexual products," the authors doubtless mean the ova and the developing embryos.

We consider this is imperative and that the conclusions Keysselitz and Mayer have come to after using caught flies are erroneous and misleading.

Roubaud (1908) has recently devoted attention to what he considers to be a special development of pathogenic trypanosomes in the proboscis of *Glossina palpalis*. In two of these flies, *naturally* infected, he found flagellates fixed in tufts on the internal surface of the proboscis channel, walls of labrum and hypopharynx; the parasites were slender, measuring from 20—22  $\mu$  in length. The intestinal tracts of the flies also contained enormous numbers of flagellates, some of which were without flagella. Roubaud was unable to infect animals with any of these parasites. As a result of his observations he concludes that there are three methods of development of vertebrate trypanosomes in the *Glossinae*:

(1) Harmless culture of trypanosomes in the posterior portion of the midgut in the residuum of the digested blood. This culture disappears as soon as the flies are allowed to starve or when they feed again on blood.

(2) Special development of trypanosomes in the salivary fluid in the proboscis which is independent of (1) and is also as transitory; Roubaud considers this to be the important development in connection with the transmission of the pathogenic trypanosomes.

(3) An active multiplication in the intestine which may end in complete infection of the gut and proboscis, the parasites behaving like true parasites, and which until now have only been observed in cases of natural infection.

We can find nothing in Roubaud's work proving conclusively that these various developmental stages have come from vertebrate trypanosomes, and in spite of what he says we cannot see any difference between the forms he observed in the proboscis of *G. palpalis* and those described by Keysselitz and Mayer in *G. fusca*. We therefore can only consider these various developmental forms as representing stages in the life-cycles of one or more natural parasites of the fly which have no connection with any vertebrate trypanosome. The very fact that Roubaud was unable to infect animals with a pathogenic trypanosome by inoculating them with the forms he has seen in the proboscis of *G. palpalis* is, in our opinion, conclusive evidence that they are natural parasites of the fly. We have no difficulty in understanding that the conditions in nature are much more favourable for the infection of the flies with these natural flagellates. The three types of infection as

outlined by Roubaud in our opinion may quite well represent the development of two distinct flagellates of *G. palpalis*; one of these may be *grayi*, which we know encysts in this fly, while the other may be that known as *tullochi*, and it is possible the latter is transmitted hereditarily. Minchin's work on the encystment of *grayi* has not been followed up, on the contrary we find this flagellate is still regarded as a vertebrate trypanosome, and nothing whatever is known of the species *tullochi*.

Before concluding these remarks on the flagellates of tse-tse flies, we would like to urge on workers who have the opportunity of studying these parasites that it is of the utmost importance to ascertain how they are transmitted from one fly to another. We know the species *grayi* encysts in the rectum of *G. palpalis*; these cysts and their further development should next be looked for in the stomachs of this fly, either before it has first fed or soon after. The infection probably takes place in nature by the flies ingesting the cysts accidentally either before they fly off to get their first feed of blood or when they return to their breeding grounds between their several feeds. One experiment of Minchin's, quoted above, shows that it is possible to infect a clean fly in captivity. Freshly hatched out *G. palpalis* (after first ascertaining that they have not got a flagellate which is transmitted hereditarily), should be put in cages in which wild flies *G. palpalis* have been kept, some of these are sure to pass out the cysts of *T. grayi* in their faeces and it could then be seen whether the clean flies become infected.

In the case of *G. fusca*, and this applies also to all the other tse-tse flies, it could be readily demonstrated whether its flagellate is transmitted hereditarily by raising flies from infected parents and then feeding them on clean animals; pupae raised from infected flies should certainly be examined. It is important to keep in mind the possibility of any species of tse-tse fly being infected with two distinct flagellates, one of which may encyst in the rectum of its host and the other be transmitted hereditarily. One of us (1908) has recently made a suggestion which we have found of great use in studying these natural flagellates, and that is to divide their life-cycles into three stages, *pre-flagellate*, *flagellate* and *post-flagellate*; although these stages are not sharply separated from each other they are sufficiently distinct to enable anyone to follow the various forms as they occur in the flies. We believe that until these flagellates are completely studied, apart from any possible developmental form of trypanosomes, the important

problem in connection with the transmission of the pathogenic trypanosomes of Africa will not be completely solved.

We now propose dealing shortly with the question of the development of trypanosomes of fishes, frogs, eels and leeches. It has been accepted as an incontestable fact that the trypanosomes, mentioned above, undergo developmental changes in leeches. The first important work on this point is that of Keysselitz (1906) who claims to have followed the development of *Trypanoplasma borreli* in *Piscicola geometra*. This investigator, recognising the fact that leeches caught at large harboured flagellates, raised them from the egg, and on feeding them on fish infected with *Trypanoplasma borreli*, he observed certain developmental changes. He however failed to infect fish either by placing leeches on them or by injecting them with the gut-contents of infected leeches. We have pointed out above that in the case of a frog leech it was found that it harboured a flagellate which was transmitted hereditarily, and that it was not possible to trace any connection between this flagellate and frog trypanosomes. In the case of this particular leech it is not possible to exclude its natural flagellates merely by raising leeches at random; it is necessary first to make certain that the parent leech has no flagellates. Keysselitz makes no mention of this, and without the rigid exclusion of a natural flagellate we cannot accept this author's developmental cycle of *Trypanoplasma* as being free from error. It is also not at all clear how *Trypanoplasma borreli* passes into the developmental forms described by Keysselitz; in fact, this is exactly similar to what we have remarked in the case of the developmental forms of trypanosomes in tse-tse flies, lice, etc. Léger (1904) has also described the development of trypanosomes and trypanoplasms in leeches, and Brumpt (1904-1908) has extended this work, describing the development of trypanosomes of fresh water fishes, eels and frogs in various leeches. He also discovered that some of these leech flagellates, particularly those found in leeches from frogs, are transmitted to the progeny of the leech. França (1907) has also recently described the development of a frog trypanosome in a leech. In every instance, and we have looked carefully through the literature, these authors never mention the possibility of these flagellates being *harmless parasites of leeches*. It would appear that in each case leeches are fed on frogs etc. infected with trypanosomes; flagellates are later found in their crop diverticula and intestines and these are then described as developmental forms of the trypanosomes. In each particular leech the transition from the vertebrate trypanosome to the so-called



*Crithidia*-like or *Herpetomonad* form is exceedingly vague and abrupt, and it should be noted that no sexual cycle appears necessary in these trypanosomes. We are therefore unable to accept these authors' results and consider all their experiments are contaminated with possible natural flagellates we have referred to above.

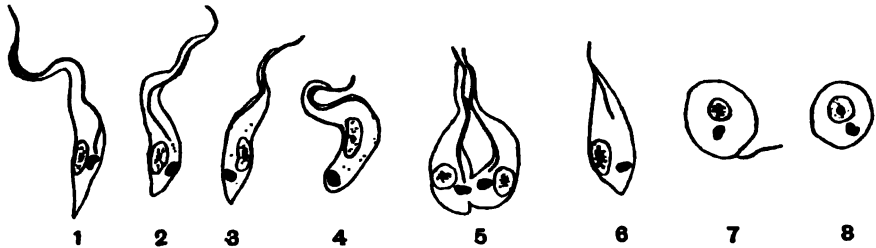
In *all* the instances in which vertebrate trypanosomes have been described as undergoing developmental changes<sup>1</sup> in invertebrate hosts, the presence or otherwise of natural flagellates has been entirely overlooked, and the work that is already recorded is of very little value. These natural flagellates are not "*cultural forms of trypanosomes*" but are true parasites of invertebrates. They have been found in *lice*, *fleas*, *mosquitoes*, *ticks*, *biting and non-biting flies*, *bugs* and in *leeches*, and we now propose recording our observations on a flagellate one of us found in the alimentary tract of *Ctenophthalmus agyrtes*. This flea, the Hon. C. N. Rothschild informs Professor Nuttall, has not been previously recorded from the rat, *Mus decumanus*, but that it is very common on mice, moles and shrews. We took a large number, as many as 16 off one rat, from a batch of animals caught at a particular locality in Cambridge. A large percentage of the rats were infected with *T. lewisi*.

*Crithidia ctenophthalmi*, n. sp.

We have examined the alimentary tracts of 25 fleas, and found two of them infected with this parasite; one of the fleas was from a rat infected with *T. lewisi* while the other flea came from a rat in whose blood we could not find any trypanosomes. The alimentary tracts of both the fleas, on being dissected out, were examined in the fresh condition; the midgut of the first contained fresh blood but no parasites could be seen moving in any part of it. The whole alimentary tract was ruptured and the contents smeared out and stained with Giemsa's stain; a number of adult flagellates and encysting forms were found in it. The midgut of the other flea contained digested blood alone, no parasites were seen in it but, on carefully examining the contents of the rectum, motile flagellates as well as round motionless forms were readily seen. The rectum was isolated and its contents smeared out and stained with Giemsa's stain. In this preparation it was possible to study the *post-flagellate* stage of this *Crithidia*. One of us (1908) has

<sup>1</sup> We do not deny the possibility of trypanosomes undergoing simple multiplication by longitudinal division; Minchin (1908) has clearly demonstrated this in the case of *T. gambiense* in *G. palpalis*.

pointed out that this stage is characterised by the flagellates collecting in the hindgut and rectum of their host, where they shorten, divide longitudinally, the flagella on degenerating are shed and the parasites finally round up into cysts.



Figs. 1—8. ( $\times 2900$ .)

In the film made from the contents of the rectum of the infected flea, noted above, we were able to recognise every stage from the adult flagellate up to the formation of the cysts. Fig. 1 represents one of the adult flagellates as yet unchanged, and it will be seen that its anterior end is prolonged along the flagellum, a short portion of which is free. The blepharoplast is a large rod-shaped structure, and in the majority of these unchanged flagellates, is seen lying either alongside the nucleus or just anterior to it. The flagellum is a single stout filament and arises in close proximity to the blepharoplast. In our preparation we have not seen any basal granule. On looking at a series of these flagellates we were struck by the fact that the blepharoplasts appear to migrate towards the posterior end so that these parasites come to look very like trypanosomes; we have depicted some of these appearances in figs. 2, 3 and 4. The explanation of this migration appears to us to be quite simple; in many of these forms it is seen that, though the flagellum is quite clearly stained at its free end, it is indistinct and hardly visible towards the blepharoplast and in some specimens this portion has quite disappeared (figs. 2, 3 and 4). We believe, therefore, that at this stage as the flagellum begins to degenerate the blepharoplast is freed and tends to pass towards the posterior end of the cell. We wish to draw attention to this peculiar appearance because it may, if studied alone, be quite well confused with a trypanosome. We have seen similar appearances in some *Herpetomonads* where the blepharoplast comes to lie behind the nucleus. At the same time as these changes are taking place, the anterior ends of the parasites become shortened

and fig. 4 shows an early stage in this process, while fig. 6 represents a more advanced stage, the anterior end having rounded up. One of us has figured and described somewhat similar changes in the post-flagellate stage of *Crithidia gerroidis*.

It is quite common to find these encysting flagellates in all stages of division, and fig. 5 shows two very nearly separated. After the anterior end is drawn up completely the flagellum is shed and in some of the parasites they are seen as tags attached to the bodies of the parasites; we have depicted this appearance in fig. 7. In fig. 8 we show the final stage of encystment; these bodies are round or oval and contain a nucleus and blepharoplast. There were only a few of these forms in the preparations, the majority of the parasites were still flagellates. In other species we have found these cysts in enormous numbers in the rectum and hindgut of their hosts. This, then, is a mere outline of a part of the life-cycle of this *Crithidia*; we do not pretend that our observations even on this stage are complete. It would be necessary to examine the parasites in the fresh condition and watch these changes taking place. Unfortunately our material has been limited so that we are not able at present to undertake an extensive study of the parasite. It will be seen from our description and figures that this flagellate corresponds exactly with our definition (see below) of the genus *Crithidia*. In its adult flagellate stage the anterior end is drawn out, and it has a rudimentary undulating membrane; we propose therefore naming it *Crithidia ctenophthalmi*. It is important to consider how the fleas become infected, and we need hardly say that this flagellate is in no way connected with *T. lewisi*. We have examined a number of fleas (*C. agyrtes*) from rats heavily infected with *T. lewisi* but have never seen any developmental changes similar to those described by Prowazek in lice. The fact that we have seen the encysted stages of this parasite in the adult flea suggests that the cysts are passed out in the faeces of the flea and are ingested again either by the adult flea or its larva. It is obvious that the larva is much more likely to ingest the cysts and one of us has found a similar *Crithidia* in the larvae of *Ctenocephalus felis*. We are at present breeding fleas (*C. agyrtes*) and hope later, should we have sufficient material, to study this flagellate completely.

Flagellates appear to have been first recorded from fleas by Balfour (1906), who found them in the hindgut of *Loemopsylla cleopatrae*, Rothschild. This parasite, as far as we can judge from Balfour's figures, is a *Crithidia*. Swingle (1907) was the next to record the occurrence of flagellates in rat fleas (species not named) in Nebraska; he at first

mistook them for developmental forms of *T. lewisi*. We cannot say whether this parasite is a *Crithidia* or a *Herpetomonas*. Lastly, one of us, as mentioned above, found a *Crithidia* in the gut of the larva of *Ctenocephalus felis* in Madras. The larvae of this flea suck the blood of the cat and wherever a tame cat used to sit larvae gorged with blood were found in large numbers.

#### *Concluding Remarks.*

It is again necessary to make some remarks on the genus *Crithidia* of Léger as we find authors are still calling certain flagellates *Crithidia* which are clearly *Herpetomonads* and *vice versa*. This genus was created by Léger in 1902 for a flagellate he found in *Anopheles maculipennis*, and the name was based on what Léger considered to be the characteristic shape of this parasite, a short truncated (barley corn) organism. One of us has shown that the genus *Herpetomonas* also has a very similar stage and that the short truncated forms of *Crithidia fasciculata* are its young stages. We have also pointed out that by following Léger's description authors have placed a true *Herpetomonas* in the genus *Crithidia*, and *vice versa*. It is only necessary to refer to Léger's (1902) original description and figures of *Crithidia fasciculata*, when it will be seen that the adult flagellate of this parasite is exceedingly characteristic, and we have never seen any *Herpetomonas* like it. Owing to the fact that observers have so far only studied stages of these parasites, it can readily be understood how errors have arisen.

A great deal of the confusion regarding these flagellates is also undoubtedly due to Prowazek's erroneous view of the flagellar apparatus of *H. muscae domesticae*. One of us has shown clearly that, apart from the study of the *flagellate* stage of *Herpetomonas muscae domesticae*, by following the *pre-flagellate* stage up to the formation of the flagellum it can be demonstrated beyond any doubt that it has a single flagellum. Where then can the double flagellum come from? One of us has studied this flagellate for the last two years, and will shortly give a complete account of it where it will be shown that a fly may be found with almost all the flagellates showing the appearance of the double flagellum, and again in another fly the majority have a single flagellum. Finally, in following the *post-flagellate* stage of *H. muscae domesticae* the parasites have been observed to have *only* a single flagellum. The subject is still more confused by the recent introduction by Chatton, Alilaire (1908)

and Roubaud (1908) of the generic name *Leptomonas* of Kent. These observers would place all the *Herpetomonad*-like flagellates with one flagellum in this genus, while those with a double flagellum are relegated to the genus *Herpetomonas*, and Roubaud states he has observed the structure of *H. muscae domesticae* as described by Prowazek; so have we, but we are unable to give any other interpretation than that given above: we do not know on what Roubaud's statement is based. In order to make our first point clear as to how observers may be led astray by studying only one stage of a flagellate we will refer to another recent paper. Werner (1908) has described what he considers to be a *Crithidia* from the faeces of *Musca domestica*; unfortunately his photographs are so indistinct that it is not possible to make out clearly the structure of the parasite. If this parasite is a true *Crithidia* it is obvious that the author has again gone back to Léger's definition of the genus, and we consider this is a serious step backwards, and that instead of helping to a natural classification it will lead to greater confusion. We note that Werner's parasite was only found in the faeces; no mention is made as to whether the rectum and hindguts of the flies were examined for flagellates, and it is not stated whether the flies were infected with *H. muscae domesticae* as well. Werner refers to the similarity between his parasite and one recently described from *Culex pipiens* (*H. culicis*) by one of us. On referring to the figures of this *Herpetomonas* we find the only forms which appear to have a slight resemblance to Werner's parasite are those depicted in figs. 9, 10 a, 10 b. For the sake of clearness the various stages of this *Herpetomonas* were figured in a circle so that each stage could be readily followed. The figures to which Werner apparently refers clearly represent the young flagellates; it is our whole contention that a genus cannot be based on these immature forms. The adult flagellate of *H. culicis* is clearly depicted, and we fail to see what resemblance there is between it and Werner's parasite. One of us has seen parasites very like those described by Werner, and we know they represent the *post-flagellate* stages of *H. muscae domesticae*. In numerous living specimens we have seen the flagellates of the house fly collecting in masses in the rectum of the insect where the typical long forms shorten, divide and eventually round up, so that we would like to know what the adult flagellate of Werner's parasite is like. Remarking on the marked difference in size between his parasite and the flagellates of *H. muscae domesticae*, Werner believes it improbable that they could be in any way connected. We would draw attention to the still more marked difference in size between the cysts of *H. muscae domesticae* and the adult flagellate,

yet they undoubtedly belong to the same parasite. Again, we would refer to the striking difference in size between the cysts of *Herpetomonas lygaei* ( $3.5\ \mu$  to  $4\ \mu$ ) and the adult flagellate which may measure as much as  $25\ \mu$ .

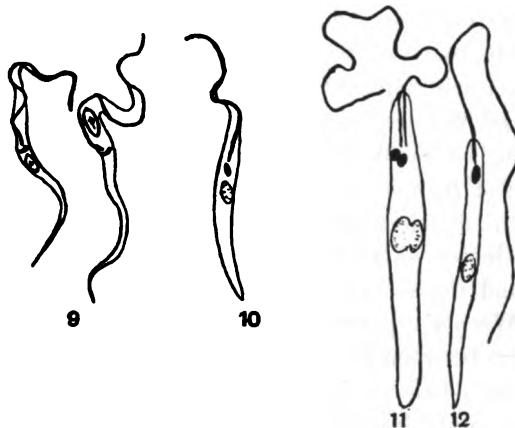
In order, therefore, to make our conception of the genus *Crithidia* quite clear we will define it as follows:—

*Crithidia* Léger, 1902 (emended by Patton, 1907).

*Flagellates which in their adult stages have a fusiform body with a blepharoplast, usually a large rod-shaped structure, situated close to the nucleus either anterior or a little distance posterior; their anterior ends are attenuated and drawn out along the flagella to which they are attached by a narrow undulating membrane, which never has the characteristic folded appearance seen in adult flagellates of the genus Trypanosoma. Their posterior ends may be blunt or pointed. They have three characteristic stages in their life-cycles: pre-flagellate, round or oval bodies with a nucleus and blepharoplast which multiply by simple fission; flagellate stage when they multiply by longitudinal division which may be either equal or unequal; in this stage they often exhibit marked polymorphism; post-flagellate stage when the flagellates shorten, divide and then encyst, some species (in ticks, leeches and *Melophagus ovinus*) pass this stage in the eggs of their hosts.*

Fig. 9 represents two adult flagellates of *Crithidia haemaphysalidis*, in one the blepharoplast is well behind the nucleus; note the pointed posterior ends. Fig. 10 is an adult flagellate of *Crithidia gerridis*.

These flagellates differ markedly from the genus *Herpetomonas*



Figs. 9—12.

which has a truncated anterior end, *single* flagellum and no undulating membrane. Figs. 11 and 12 are two flagellates of *Herpetomonas muscae domesticae* from the same fly; fig. 11 shows the appearance of the double flagellum, which we regard as the early stage in division, and fig. 12 represents an adult flagellate with a single flagellum.

The *Crithidia* are closely allied to the *Trypanosoma* in that they possess a rudimentary undulating membrane, and in some species, particularly those occurring in ticks (fig. 9), the blepharoplast passes behind the nucleus; many of these forms however represent early division stages (unequal) and in the typical adult forms the blepharoplast is never very far behind the nucleus.

These flagellates have up to the present only been found in the alimentary tracts and malpighian tubes of arthropods and leeches, and it is a remarkable fact that the majority of known species occur in blood-sucking invertebrates. In another paper in this *Journal* (p. 314), Wenyon records a very interesting flagellate from the blood of *Erythrolamprus aesculapii*; this parasite is the first flagellate we know from the blood of a vertebrate which at once suggests a *Crithidia* rather than a true trypanosome. A glance at Wenyon's figures will show that his flagellate differs considerably from the pathogenic trypanosomes of mammals. It is not at all unlike the flagellate from *Haemaphysalis flava*, which we believe is a *Crithidia*, both having attenuated posterior ends. It is impossible, however, to come to a definite conclusion regarding the exact position of the snake flagellate until more is known of its life-cycle.

We would pass the same remark on Chatton and Alilaire's (1908) parasite from the malpighian tubes of *Drosophila confusa* which they call *Trypanosoma drosophilae*. It is dangerous to form a definite opinion as to the biological position of these insect flagellates on morphological grounds alone and we cannot too strongly draw attention to the fact, that a knowledge of the complete life-cycle of a protozoön is of the greatest value, for it is only then possible to form an adequate conception of any species and its relationships. We have pointed out above that in a certain stage in its life-history a *Crithidia* may be very like a young trypanosome, e.g. a short form of *T. dimorphon*. We would also like to draw attention to the great tendency there is at the present time of theorising on the origin of the trypanosomes and allied flagellates. Prowazek (1904, 1905), Brumpt (1908), Minchin (1908), Woodcock (1906), and others have each advanced a particular hypothesis of their own, and now we find Chatton and Alilaire (1908)

bring forward another on the polyphyletic origin of the *Trypanosomatidae*. Is the evidence to hand sufficient to justify this hypothesis?, we think not. Chatton and Alilaire have only observed one stage of *T. drosophilae*, and we are not even told whether this is the adult flagellate stage, so that it is difficult to accept the authors' contention that this parasite is a true trypanosome.

*In the present state of our knowledge it is imperative that we should obtain accurate and detailed descriptions of the life-cycles of these flagellates of arthropods and leeches. It is also essential to determine the manner in which infection occurs. We may then be in a position to theorise on the structure and origin of the Trypanosomatidae and other allied forms.*

In the meantime we propose placing the following species in the genus *Crithidia* :—

1. *Crithidia fasciculata* Léger, type species parasitic in the alimentary tract of *Anopheles maculipennis*. Only a part of the life-cycle of this parasite is known. Mezincesco (1908) in a recent paper refers to this flagellate as *Trypanosoma culicis* Novy (1907); *Crithidia fasciculata* of Novy, MacNeal and Torrey is obviously *Herpetomonas culicis*.

2. *Crithidia campanulata* Léger, parasitic in the alimentary tract of *Chironomus plumosus*, life-cycle not known.

3. *Crithidia minuta* Léger, parasitic in the intestine of *Tabanus tergstinus*, life-cycle not known.

4. *Crithidia subulata* Léger, parasitic in the alimentary tract of *Tabanus glaucopus* and *Hematopota italica*, life-cycle not known.

5. *Crithidia gerridis* Patton, parasitic in the alimentary tract of *Gerris fossarum*, life-cycle recently described (Patton).

6. *Crithidia tabani* n. sp. Patton, parasitic in the alimentary tract of *Tabanus hilarius*, life-cycle will shortly be described in *Archiv für Protistenkunde*.

7. *Crithidia grayi* Novy, parasitic in the alimentary tract of *Glossina palpalis* and possibly other *Glossinae*, life-cycle partly known (Minchin).

8. *Crithidia tullocki* Minchin, parasitic in the alimentary tract of *Glossina palpalis* and possibly other *Glossinae*.

9. *Crithidia* sp.?, parasitic in the alimentary tracts of *Glossina fusca* and *Glossina morsitans*, part of the life-cycle described by Stuhlmann and Koch.

10. *Crithidia melophagia* Flu, parasitic in the alimentary tract of



*Melophagus ovinus*, life-cycle shortly to be described by Swingle, part has been described by Flu (1908).

11. *Crithidia christophersi* Novy, parasitic in the alimentary tract of *Rhipicephalus sanguineus*.

12. *Crithidia haemaphysalidis* n. sp. Patton, parasitic in the alimentary tract of *Haemaphysalis flava*.

13. *Crithidia robertsoni* n. sp. Patton, parasitic in the crop diverticula and intestine of *Pontobdella muricata*, part of life-cycle described in detail by Robertson (1907), method of infection not clear; it is believed by Brumpt and Robertson to be connected with *T. rajae*, this is however not proved.

14. *Crithidia ctenophthalmi* n. sp. Patton and Strickland, parasitic in the alimentary tract of *Ctenophthalmus agyrtes*, part of the life-cycle described. Other unnamed species have been found in *Loemopsylla cleopatrae* and *Ctenocephalus felis*.

15. *Crithidia haematopini* n. sp. Patton, parasitic in the alimentary tract of *Haematopinus spinulosus*, part of the life-cycle has been described by Prowazek.

It is impossible to include in this list doubtful species such as *T. drosophilae*; the flagellate found in the ovaries of *Chrysops dimidiatus* by Ziemann (1905) which may be but a stage of a parasitic flagellate and another found by Léger in *Nepa* and named *Otomonas tremula*.

It is also impossible to include many more which have been found in bugs by Donovan in Madras, by Léger, Brumpt, Leydig and others in leeches, and by Leydig in ticks; it is possible that some of the flagellates described by Léger as *Herpetomonas* may eventually have to be placed in the genus *Crithidia*.

In conclusion we wish to thank Professor Nuttall for his kindness in helping us to complete this work, and we hope that the critical remarks made in this paper may be of some use in guiding subsequent workers to a better understanding of these parasitic flagellates of arthropods and leeches.

#### APPENDIX BY CAPTAIN PATTON.

A recent paper by Mesnil and Brimont entitled "Sur un Hématozoaire nouveau (*Endotrypanum*, n. gen.) d'un Edenté de Guyane" (*Compt. Rend. Soc. Biolog.* T. LXV. No 35, 11th Dec. 1908, pp. 581-583, 7 figs.) calls for some remarks. The parasite here recorded was apparently found by Brimont in the red blood corpuscles of the common

Edentate, the two-toed sloth, *Cholaepus didactylus* (Linn.) in St Laurent du Maroni. The parasite measures from 8—11  $\mu$  in length and from 2·5—4  $\mu$  in breadth, it is rounded at one end and pointed at the other, it is not pigmented. When stained by Giemsa's stain it is seen to contain a circular nucleus and a well defined rod-shaped blepharoplast either lying beside the nucleus, anterior or posterior to it. The pointed end is described as the anterior and although in some of the parasites it is filamentous no flagellum could be demonstrated. The invaded corpuscles are not hypertrophied, nor do they contain any granules; they are simply deformed owing to the shape of the parasite. In the majority of the corpuscles only one parasite was found but in one there were two. Two elongated bodies, possibly parasites, were seen in a mononuclear leucocyte.

The authors remark that this intracellular parasite recalls the *Haemocytozoa*, for example the haemogregarines, in that it invades a red blood corpuscle, is elongated and not pigmented. They consider it constitutes an intermediate type between the *Trypanosomata* and the *Haemocytozoa*, like *Leishmania*, since it shows in the blood of a vertebrate host all the morphological characters of a true flagellate as *Leishmania* shows in cultures (*sic*) and in *Cimex rotundatus*. Mesnil and Brimont believe that this new parasite is more closely related to *Trypanosoma* than to *Leishmania*, and, as it invades the red blood corpuscles of a vertebrate, they have created for it a new genus, *Endotrypanum*.

From an examination of the authors' figures of the stained specimens it is clear that the parasite does not possess a flagellum; it therefore cannot have all the morphological characters of *Herpetomonas donovani* as seen in cultures and in *Cimex rotundatus*; I have clearly shown that the parasite of Kala Azar is a *Herpetomonas* and not a *Haemocytozoon*. It is however certain that the parasite of the sloth represents a stage in the life-cycle of a flagellate, probably a part of the *post flagellate* stage. The question naturally arises, how does it invade the red blood corpuscles? The shape of the parasite certainly suggests that it may penetrate them when possessing a flagellum which later disappears. No free forms, similar to those in the corpuscles, were seen. It is possible this intraglobular stage is a transitory one and that the parasite undergoes other changes in the organs of the sloth. The rounded binucleate stage should certainly be searched for in the leucocytes and endothelial cells as well as in the transmitting invertebrate.

The structure of this organism suggests that it is closely allied to the *Crithidia* as defined above. These flagellates have so far only been

found in invertebrates, and, as we now know, three Herpetomonads which pass a part of their life-cycles in a vertebrate, and a large number of trypanosomes which only live in the blood plasma of vertebrates, there seems no reason why the closely allied *Crithidia* should not be found also in the blood of vertebrates. *Crithidia* are common in blood-sucking invertebrates and we know of some that are well able to penetrate the ova of their hosts, so that there is no reason why they should not be able to penetrate red blood corpuscles. I am unable to follow the authors when they say that the discovery of this parasite supports Schaudinn's view on the phylogenetic relations of *Trypanosoma* and *Haemocytosoma*. There is no evidence to show that the parasite is a *Sporozoon*. The fact that it invades the red blood corpuscles of a vertebrate merely indicates that it is a highly specialised flagellate; cf. the mammalian leucocytozoa, *L. canis*, *L. funambuli*, etc. The occurrence of a flagellate in the corpuscles of a vertebrate further emphasizes the fact that we know very little about these parasitic flagellates and that without a complete knowledge of all the forms and their life-cycles it is futile to construct hypotheses. The discovery of further stages in the development of this new flagellate will be awaited with great interest.

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ON THE STRUCTURE OF THE SPIRACLES OF A TICK—  
*HAEMAPHYSALIS PUNCTATA*, CANESTRINI AND FANZAGO.

By G. H. F. NUTTALL, F.R.S., W. F. COOPER, B.A.

AND L. E. ROBINSON, A.R.C.Sc.

Plates XXII, XXIII.

THE detailed structure of the spiracles in the Ixodoidea has hitherto received little or no attention at the hands of zoologists; at the same time, these organs are sufficiently extraordinary to make it a matter of surprise that, so far as our knowledge of the literature goes, not one of the numerous contributors to the subject of tick anatomy has found it worth while to undertake a complete description or to publish figures to illustrate it. Batelli (1891) gives a short account of the structure of the spiracle of a tick, presumably *Ixodes ricinus*, with a single figure, but with this exception we have been unable to find any further information on the subject.

The material for this communication has been accumulated in the course of our work on the anatomy of ticks, and in view of the interesting features presented by the spiracles, we have preferred to publish a separate account of these structures, although a more or less general description will appear later in the second part of our paper on the anatomy of *Haemaphysalis punctata*<sup>1</sup>.

The spiracles are situated in the posterior half of the body, on the lateral margins, towards the ventral surface, immediately behind and a little external to the coxae of the fourth pair of legs. Each consists of a slightly elevated "plaque" with a well-defined margin. The dimensions of the spiracle show a considerable amount of variation; in the adult tick the length of the spiracle usually exceeds the breadth, in the nymph the reverse is the case. The following table gives the

<sup>1</sup> See this volume, pp. 152—181.

actual dimensions in both sexes and the nymphal stage, measured on ten specimens of each taken from a large number collected in Kent.

*Dimensions of Spiracle in Haemaphysalis punctata.*

(In microns.)					
Male		Female		Nymph	
Length	Breadth	Length	Breadth	Length	Breadth
510	320	500	430	160	170
500	310	460	410	160	160
500	310	430	360	160	150
500	300	410	400	150	170
490	350	400	360	150	170
480	330	390	360	140	160
470	320	360	350	140	150
460	270	350	320	140	150
440	260	340	320	130	150
430	270	330	330	130	140

The contour of the spiracle differs in the three cases, although in the case of the male an approach in shape to the female type may be frequently observed. The spiracle of the female (Pl. XXII, fig. 1) is more or less angular in outline, this being due to a flattening of the contour of the lateral margin; that of the male has a more or less rectangular figure and becomes gradually narrower towards its posterior portion. The spiracle of the nymph is almost circular, with a slight rounded angle at the postero-lateral margin.

The surface of the spiracle is slightly concave with the exception of a small central area, the macula (Pl. XXII, figs. 1 and 2), dark in colour and slightly eccentric in position, situated a little towards the antero-mesial margin. The macula is elliptical in outline and placed obliquely with the major axis inclined at an angle of about 45° to the median axis of the body, the anterior end being directed outwards. The marginal portion of the spiracle is formed of dark-coloured chitin, and the area between this margin and the macula, of a pale greyish-yellow colour, is perforated by numerous regularly-distributed minute pores (*ep.*); these are best seen by examination of uncleared specimens in reflected light. A limited number of coarser pores open on the marginal portions of the plate (*mp.*). The macula of the spiracle of the female is cleft by a large slit-like opening which we have termed the ostium (Pl. XXII, figs. 1, 2 *os.*); the extremities of the latter are curved round in a crescentic manner with a convexity towards the antero-mesial margin of the spiracle. It is bounded on its external



side by a prominent raised lip (Pl. XXIII, figs. 5—7), which overhangs the opening and effects its closure when the lip is depressed by muscular action. The ostium does not appear in the male or nymph<sup>1</sup>.

It is only when cleared and mounted specimens of the entire spiracle are examined that its complex structure becomes apparent. That which on examination *in situ* appears to be a simple sieve-like plate, resolves itself into a series of three superposed layers, each differing from the other two in structure. The superficial layer exhibits a regular reticulate pattern which, as will be seen later, is due to a thickening of the chitin on its under surface; the meshes of this reticulum are more or less circular, and each is perforated in its central portion by one of the minute pores (*s.p.*) already alluded to. It is almost impossible to define these pores in cleared entire preparations of the spiracle on account of their extremely thin margins being obscured by the underlying parts. Immediately beneath the superficial layer is a large air space, traversed by an arrangement of innumerable delicate chitinous rods or pedicels (*p.*), the slightly expanded bases of which arise from a thick basal layer of chitin, the latter forming the deepest layer and the most substantial portion of the spiracular plate. The basal layer, like the superficial layer, is porose, but its pores (*i.p.*) are not pervious to air, being occupied by protoplasmic extensions from the hypodermal cells underlying the spiracle. The internal pores coincide in position with the superficial pores and are those seen in ordinary cleared and mounted preparations. The intermediate or pedicellar layer is formed by the system of pedicels which support the superficial layer. The arrangement of the pedicels shows a regular system; this is readily seen in Pl. XXII, figs. 2 and 4. The pedicels are triangular in cross section and are slightly curved in such a manner that their upper extremities are brought together and fuse with one another on the under surface of the superficial layer; it is this fusion of the pedicels which forms the reticulate thickening of the superficial layer.

By the study of a series of vertical transverse and longitudinal sections of the spiracle, it becomes possible to interpret the relationship of the parts described in the preceding paragraph (Pl. XXII, fig. 3 and Pl. XXIII, figs. 1—10). The continuity of the thin superficial layer is

<sup>1</sup> The terms macula and ostium are both new: the term pore has been very loosely used in general descriptions and appears to refer to the macula. We see, however, that the macula may contain no pore or aperture communicating with the tracheal system.

broken at intervals by the external pores (*e.p.*) and directly beneath the latter are seen the internal pores (*i.p.*) passing through the basal layer. Large pyriform air spaces are formed between the groups of pedicels beneath each of the external pores, and these pyriform spaces (*p.s.*) establish direct communication between the two series of external and internal pores.

The portion of the spiracle beneath the macula shows an entirely different structure. It is occupied by a columnar mass of connective tissue and muscle fibres running upwards from the soft structures underlying the spiracle; this mass of soft tissue, which we have termed the columella, extends up to the under surface of the superficial layer which is considerably thickened at this central portion (Pl. XXIII, figs. 7—10 *col.*). Completely encircling the columella is a large annular air space, the pericolumellar space (*p.c.s.*) which communicates directly with the interstices between the pedicels in the intermediate space. The ostium opens into the pericolumellar space on the internal side of the columella (Pl. XXIII, figs. 5—7) and immediately beneath the ostium the cavity of the pericolumellar space is continued downwards as a large chamber of irregular shape which we have termed the atrium (*a.*). The cavity of the atrium is roughly elliptical in cross section, somewhat contracted dorso-ventrally; its direction is at first horizontal or slightly upwards, it then bends downwards and terminates abruptly, the entire length being equal to about half the diameter of the spiracle. Numerous main tracheal trunks (*tr.*) open separately from the basal portion of the atrium, running inwards and downwards for a short distance as a stout bundle of tubes with spirally thickened walls, from which the tracheae radiate to all parts of the body. Running up the columella and inserted along the dorsal wall of the atrium (to the right of the atrium in the figures on Pl. XXIII) is a band of muscle fibres, a small portion of which runs up to the superficial layer and is inserted beneath the centre of the macula. The contraction of this columellar muscle would dilate the cavity of the atrium and possibly at the same time close the ostium, the external margin of which overhangs the slit-like opening. This would cause the inspired air to filter through the external pores of the superficial layer. The expulsion of air from the tracheal system is effected by the contraction of the dorso-ventral body muscles which squeezes the contained air out through the spiracle, a fresh supply being inspired by the elastic rebound of the tracheal tubes, when the action of the body muscles is relaxed.



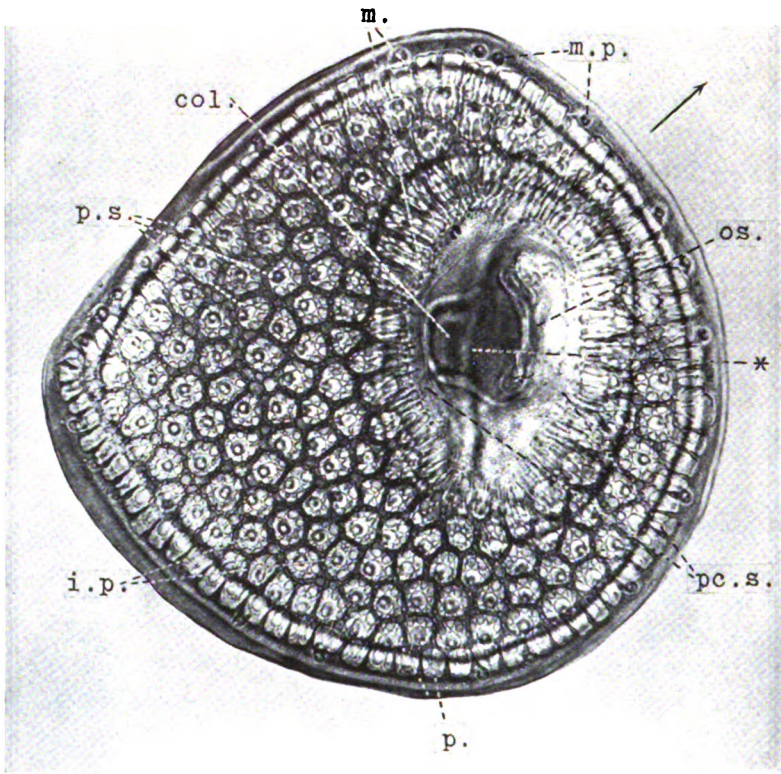


Fig. 1.

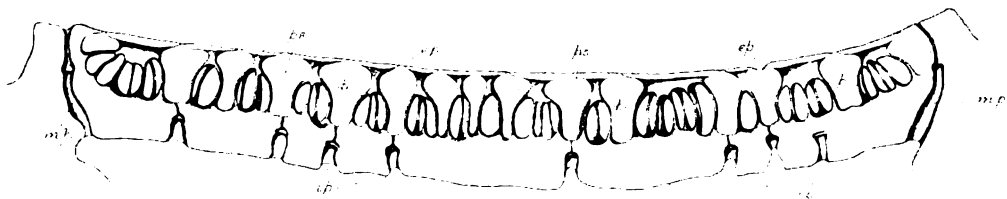


Fig. 3.

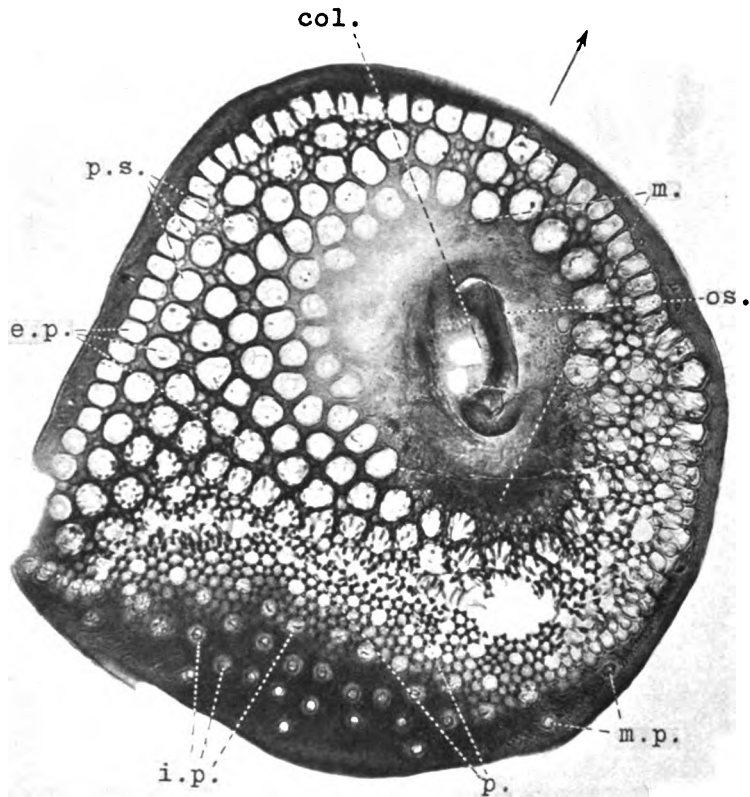


Fig. 2.

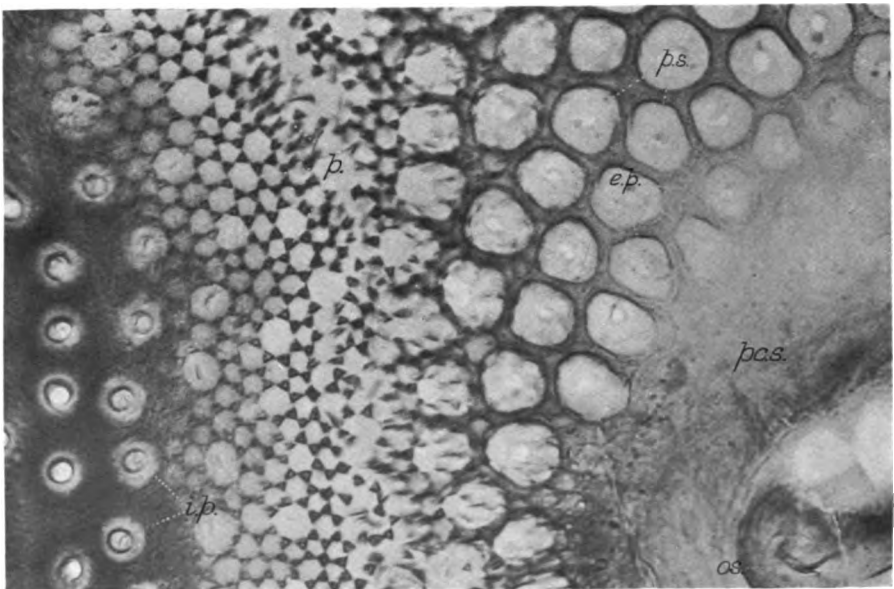


Fig. 4.



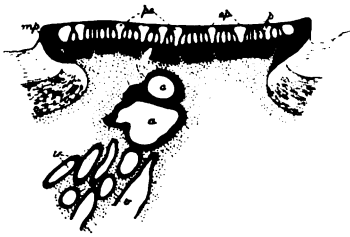


Fig. 1.

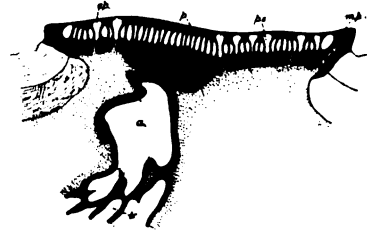


Fig. 2.



Fig. 3.



Fig. 4.

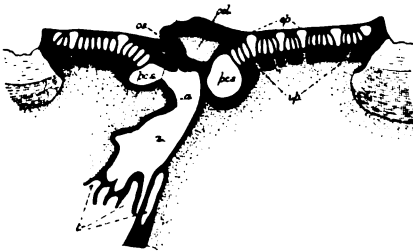


Fig. 5.



Fig. 6.



Fig. 7.



Fig. 8.



Fig. 9.



Fig. 10.





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## DESCRIPTION OF PLATES XXII—XXIII.

### PLATE XXII.

- Fig. 1. *Haemaphysalis punctata* ♀. Right spiracle as seen in transmitted light; the arrow indicates the direction of longitudinal axis. Photomicrograph (L. E. B.) × 220.  
 Fig. 2. *Haemaphysalis punctata* ♀. Horizontal section through right spiracle passing in a slightly oblique plane and cutting the different layers successively; the arrow indicates the direction of the longitudinal axis. Photomicrograph (L. E. B.) × 220.  
 Fig. 3. *Haemaphysalis punctata* ♀. Transverse section through spiracle (not passing through macula) showing characters of external, internal and marginal pores. (L. E. B. del.) × 300.  
 Fig. 4. Small portion of Fig. 2, highly magnified, showing regular arrangement of pedicels, etc. Photomicrograph (L. E. B.) × 500.

### PLATE XXIII.

Chitinous structures of spiracle—black: general cuticle of body—line-shaded: soft tissues—stippled: air spaces—clear.

- Figs. 1—8. *Haemaphysalis punctata* ♀. A series of transverse sections passing through the central portion of the spiracle, showing communications between the various air spaces, the atrium and tracheae, etc. × 100.  
 Figs. 9—10. *Haemaphysalis punctata* ♀. Longitudinal sections passing through the macula on external side of ostium, Fig. 10 being the more internal of the two. × 100.

## INDEX TO LETTERING ON PLATES XXII—XXIII.

a.	atrium.
col.	columella.
e. p.	external pores.
i. p.	internal pores.
m.	macula.
m. p.	marginal pores.
os.	ostium.
p.	pedicels.
p. s.	pyriform spaces.
pc. s.	pericolumellar space.
tr.	tracheae.

\* (Pl. XXII, Fig. 1) indicates external wall of atrium, seen through thickness of spiracular plate.

The arrows on Pl. XXII, Figs. 1 and 2 indicate the direction of the longitudinal axis of the spiracle.

A CONTRIBUTION TO THE LIFE HISTORY OF  
*ECHINOSTOMUM SECUNDUM*, NICOLL.

By MARIE V. LEBOUR, M.Sc.

*Assistant Demonstrator in Zoology, Leeds University.*

Plate XXIV.

IN some notes on the Trematodes of Northumbria published in 1905 a few remarks were made on a larval Trematode inhabiting the liver of the common periwinkle *Littorina littorea*. The liver in two per cent. of the periwinkles from Budle Bay was full of rediæ containing cercariæ more or less developed, the latter agreeing in every way with an encysted *Echinostomum* larva which inhabits mussels, cockles and other bivalve mollusks in the same locality. So close was the resemblance that I had no hesitation in declaring them to be the same worm in different stages, but hoped for an opportunity of demonstrating this by experiment. In October 1908 through the courtesy of Professor Meek I had the opportunity of conducting some feeding experiments in the Dove Marine Laboratory, Cullercoats, which have given satisfactory results, and although it is not possible to state absolutely that the forms are identical yet the evidence is so strong that I think I am justified in regarding the young worm in the periwinkle as an earlier larval form of the encysted worm in the foot of the mussel and cockle.

The youngest stage of this species I have seen is the redia, and I have never noticed miracidia or sporocysts. The liver in the infested periwinkles instead of being of a greenish-brown colour, as it usually is in healthy specimens, is a pinkish orange. On examination this is seen to be due to an enormous quantity of rediæ packed together. So crowded are they that very little liver substance is left and almost the whole of the spire of the shell is occupied by the worms. The full-grown redia is easily seen with the naked eye as a pinkish-yellow sac

about 2.6 mm. long and 0.48 mm. broad. Young rediæ are also present measuring about 0.40 mm. long, or, when extended, about 0.60 mm. These young rediæ are very active and move continually by alternate contraction and extension. They are quite colourless and transparent, have a large muscular oval sucker leading into a strongly developed pharynx and an inconspicuous intestine containing no food material. (See Plate XXIV, fig. 1.) The anterior end of the body is marked with broad wrinkles and this part terminates posteriorly in a "collar." At the tail end are two blunt processes which disappear in the full-grown rediæ. The body is full of masses of cells which are evidently the beginning of the formation of the cercariæ.

The redia at a later period begins to feed, and, as it grows larger, food material is seen in its sac-like intestine as yellow and brown granules (black in the figure). The body becomes pinkish or orange, the striations, collar and posterior appendages disappear; the shape is now simpler and somewhat resembles that of a stocking and the creature is very inert (Plate XXIV, fig. 2). Inside the sac are many cercariæ in various stages of development, and, when full-grown, these break out of the redia at the posterior end.

The cercaria is colourless and transparent and consists of three parts, (1) a heart-shaped head, (2) an elongated body, (3) a very active tail, not quite so long as the body. The length of the worm minus the tail is about 0.70 mm. The tail lashes continually backward and forward and may be occasionally seen detached from the body and moving about in the redia. When the worm loses its tail it moves in a leech-like manner by means of its suckers.

The body is covered, except at the posterior end, with minute spines and the head bears 29 large pointed spines arranged in an incomplete circle. The three last spines on each side are arranged in a peculiar way, one large spine occurring between two which are much smaller than any of the others. These two spines are on a lower level.

The oral sucker is at the extreme anterior end and is much smaller than the ventral which is situated well behind the centre of the body. The mouth leads into a long narrow prepharynx followed by a muscular pharynx and a short œsophagus which divides near the centre of the body into two narrow intestinal lobes; the latter reach to the posterior end of the body. The excretory system consists of an oval posterior vesicle into which open two very much branched lateral ducts which begin one on each side of the oral sucker, curve gently inwards in a small bay and then receive the lateral branchlets. The ducts are

full of highly refractive granules and are the most conspicuous organs in the worm.

A great part of the body is taken up by gland cells. Two straight ducts run forward from these, one on each side of the oral sucker and split anteriorly into smaller ducts which open just in front of the oral sucker. These glands are probably used for the secretion of the cyst since they disappear in the encysted condition (Plate XXIV, fig. 3).

The cercaria is now ready to leave its first host the periwinkle. How it gets out has not been observed. Possibly it enters the alimentary canal and issues from the anus; or it may bore its way through the tissues of its host, or the periwinkle may die and so liberate the cercariæ. The last hypothesis is the least likely as the cercariæ do not all grow up at the same time, sometimes only one or two are ready to leave the periwinkle at one time and sometimes a great many reach this stage at once.

It may be presumed that after leaving the periwinkle the tailed cercaria swims about in the water until it finds its second host in which it settles down and encysts.

The second or intermediate host is the mussel. This is practically proved by the experiments given below. The same encysted cercaria has been found in the foot of *Cardium edule*, *Mya arenaria* and *Tapes pullastra*. The above description exactly corresponds to the encysted cercaria except that in the latter the tail and glands have disappeared. The cyst is thin-walled and measures 0.2—0.25 mm. across. It is clear and colourless and is easily burst by slight pressure.

The encysted stage of this worm was first briefly and incompletely described by myself (1904) from the cockles at Budle, and later, more fully, by Dr W. Nicoll (1906 a) from the cockles and mussels at St Andrews. Afterwards it was found abundantly in both cockles and mussels at Budle. It occurs occasionally in the liver of *Cardium edule* and *Mytilus edulis* as well as in the foot; Nicoll has also observed it round the mantle edge of *Cardium edule*.

Almost every mussel at Budle is infested with this parasite and about ten per cent. of the cockles. The enormous number of the cysts points to a common shore animal being the first host and—the periwinkle abounds in Budle Bay.

The cercaria probably enters the mussel or cockle by the mouth and bores its way into the soft parts of the body. The presence of the cysts in the liver suggests this. Those in the mantle edge may perhaps enter through the epidermis but it seems unlikely that they penetrate

the foot by boring inwards from the outer wall. The epidermis of the foot is very tough and it would probably be difficult for the worm to enter this way.

The cyst is colourless, quite transparent and possesses a thin wall. The cercaria enclosed within the cyst has now lost its tail and head glands but in other respects exactly corresponds with the cercaria from the periwinkle. The spines, suckers and excretory system can be seen through the cyst. The excretory system is very conspicuous.

Nicoll (1906 *a*, 1906 *b*) has shown that the encysted cercaria is in all probability the larval stage of a new species of *Echinostomum* which he names *E. secundum*. This worm occurs in the Oyster Catcher (*Hæmatopus ostralegus*), the Herring Gull (*Larus argentatus*) and the Black-headed Gull (*Larus ridibundus*). It has been found by Nicoll in the intestines of these birds in all stages from small young specimens, agreeing exactly with the encysted cercaria from the mussel, to adults of various lengths, the longest measuring 7.3 mm. Although *E. secundum* resembles *E. leptosomum* Creplin in many ways, it is certainly a distinct species. I have found the latter worm in the intestine of the Dunlin (*Tringa alpina*) and the Turnstone (*Streptopelia interpres*) and, although the specimens were not in a very good state of preservation, it was easy to see that they were certainly distinct from the worm described by Nicoll. Moreover *E. leptosomum* agrees with the encysted larva from *Scrobicularia tenuis* and earlier stages from *Paludetrina stagnalis* while *E. secundum* agrees with the cercaria from the mussel and periwinkle above described. As I have shown (1907) the rediæ of the two species differ in form. The cercariæ agree in almost every point except in size and in the fact that the head spines, although equally numerous in both species (29), in *E. leptosomum* are all almost alike in size. The same difference is seen in the adults and it is constant. *E. secundum* is also much broader compared with its length than *E. leptosomum*, the oral and ventral suckers are larger and the ova are of a much greater size.

We may, I think, look upon the larval forms from the mussel and cockle as the young of *E. secundum* although this is unfortunately difficult to prove. The above-mentioned birds eat the mollusks containing the cysts. The cysts dissolve in the stomach and pass into the intestine where they grow into the adult forms. The Oyster Catcher, which is common at Budle, feeds constantly on the mussels, the stomachs being nearly always full of broken pieces of the shells. The Herring Gull and Black-headed Gull are also common shore feeders at Budle.

Although infection experiments on the birds are almost impossible, I have been able to prove the infection of the mussel from the periwinkle. In experiments of this kind there is always a risk of the second host being already infected, but in this case great care was taken and a control experiment carried on at the same time.

#### *Experimental Infection.*

Two tanks A and B were used for the experiment. Sea water from Cullercoats Bay ran into these continually. The water was evidently free from the parasite as this worm has never been found at Cullercoats and almost certainly does not occur there.

The mussels (350) were procured from Blyth Harbour. They were specially gathered from wood-work and piers at least fifteen feet from the ground and from a locality where the mussels have never been known to harbour the parasite. Of these mussels, 50 were opened and examined microscopically and were found to be free from the parasite. The remaining 300 were divided into two portions, one of which was put into Tank A and the other into Tank B. Into Tank B were also put 300 periwinkles from Budle Bay, and one periwinkle which had been cracked open to show that it contained the parasite. These experiments were started on October 19th, 1908. Mud and a little seaweed from Cullercoats Bay were from time to time put into both tanks.

At intervals of about a week mussels from each of the tanks were forwarded to me at Leeds for examination, with the result that out of 30 mussels from each tank, 24 from Tank B contained the encysted worm but none of those from Tank A were infected. The number of cysts in each infected specimen varied from one to five and all were in the foot with one exception when the parasite occurred in the liver. As the mussels of the control experiment in Tank A were in no case infected it seems clear that the source from which the parasites came was the periwinkles, and that the *Echinostomum* encysted in the mussel's foot is a later larval stage of the cercaria contained in the periwinkle. The life history of *Echinostomum secundum* may therefore be summed up as follows:—

First Host	Second or Intermediate Hosts	Final Hosts
<i>Littorina littorea</i>	<ul style="list-style-type: none"> <li><i>Mytilus edulis</i></li> <li><i>Cardium edule</i></li> <li><i>Mya arenaria</i></li> <li><i>Tapes pullastra</i></li> <li><i>Macra stultorum</i></li> </ul>	<ul style="list-style-type: none"> <li><i>Hæmatopus ostralegus.</i></li> <li><i>Larus ridibundus.</i></li> <li><i>L. argentatus.</i></li> </ul>

A detailed list of the experimental mussels showing the number opened and the parasites contained in them is here given.

*Table showing the number of mussels examined and the Trematodes they contained.*

Date	Tank B		Tank A	
	No. of mussels examined	Trematodes found	No. of mussels examined	Trematodes found
30 Oct.	6	<ul style="list-style-type: none"> <li>a. 0</li> <li>b. 0</li> <li>c. 0</li> <li>d. 0</li> <li>e. 1 in liver</li> <li>f. 1 in foot</li> </ul>	6	0
6 Nov.	5	<ul style="list-style-type: none"> <li>a. 0</li> <li>b. 1 in foot</li> <li>c. 2 "</li> <li>d. 2 "</li> <li>e. 3 "</li> </ul>	5	0
18 Nov.	6	<ul style="list-style-type: none"> <li>a. 2 in foot</li> <li>b. 2 "</li> <li>c. 3 "</li> <li>d. 3 "</li> <li>e. 4 "</li> <li>f. 5 "</li> </ul>	6	0
26 Nov.	6	<ul style="list-style-type: none"> <li>a. 3 in foot</li> <li>b. 1 "</li> <li>c. 2 "</li> <li>d. 0</li> <li>e. 1 in foot</li> <li>f. 1 "</li> </ul>	6	0
8 Dec.	6	<ul style="list-style-type: none"> <li>a. 5 in foot</li> <li>b. 5 "</li> <li>c. 5 "</li> <li>d. 3 "</li> <li>e. 3 "</li> <li>f. 1 "</li> </ul>	6	0

My best thanks are due to Miss A. M. Carr who has kindly looked after the experiments and forwarded specimens to me from time to time. I am likewise indebted to Captain G. J. Robinson, Harbour Master at Blyth, who kindly procured me the mussels.

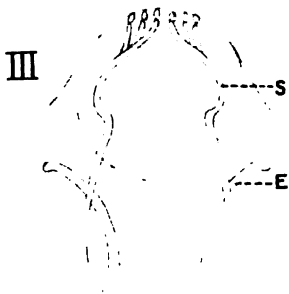
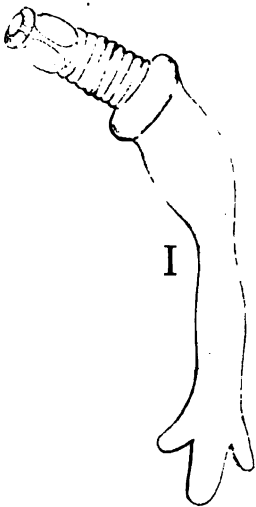
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## EXPLANATION OF PLATE XXIV.

- Fig. 1. Young redia of *Echinostomum secundum*.  
Fig. 2. Full grown redia containing Cercariæ.  
Fig. 3. Front of head of Cercaria. (*S*=ducts of glands, *E*=excretory duct. The head spines are omitted for clearness.)







**FILARIA VOLVULUS LEUCKART, ITS DISTRIBUTION,  
STRUCTURE AND PATHOLOGICAL EFFECTS.**

By ALLAN C. PARSONS, W.A.M.S., M.R.C.S. Eng., L.R.C.P. Lond.

*Med. Officer Northern Nigeria.*

*(From the Helminthological Laboratory, London School of  
Tropical Medicine.)*

(One Figure.)

ALTHOUGH *Filaria volvulus* was first described by Leuckart in 1893, very little was known or taught of this particular nematode when, ten years later, I became a government official in Northern Nigeria. While other members of the Filaridae have received a good deal of attention during the present decade, *Filaria volvulus* seems to have been comparatively ignored. I am inclined to think, however, that this *Filaria* is far more common in certain parts of Africa than is generally supposed; and, by publishing the cases that have come under my notice, I hope that something may be added to our knowledge of the obscure disease it causes and that other workers will become interested in the subject. Up to the present time I have seen five cases, and all of these came under observation at Lokoja—a large government station in Northern Nigeria, situated at the confluence of the Niger and Benue rivers. To my knowledge this is the first time that cases of *Filaria volvulus* have been reported from Northern Nigeria.

*Literature.* To Leuckart belongs the honour of first describing and naming this filaria. His material was supplied him, by a German Missionary, from the Gold Coast. Six years later Labadie-Lagrave and Deguy (1899) described a young female worm found in the case of a French soldier who had been quartered in Tonquin. Subsequently Brumpt (1904) added to our knowledge of this worm: he also considered that *F. volvulus* is a common parasite in certain inland districts of

Africa. Previously to this, Prout (1901) had given a detailed description of two worms found in a native of Sierra Leone. Finally Fülleborn (1908) wrote a critical article, illustrating the text with some interesting photomicrographs and diagrams. Besides contrasting his observations with those of Prout and others, this writer is inclined to think that the Cameroons has supplied him with a new species of filaria.

*Occurrence.* It will be noticed at once that Africa has supplied all the cases of *F. volvulus* hitherto reported. Moreover, with the exception of four cases recorded by Cooke in Uganda, the distribution appears to be limited to Western Tropical Africa. My own experience, too, corresponds with that of other observers, who lay stress on the fact that the subjects of *F. volvulus* are usually inhabitants of riverine districts. Fülleborn (1908), on the authority of Kulz, states that in the valley or district of the Wuri about 10% of the men are infested. This would seem to show that *F. volvulus* is a common parasite in certain parts of the Cameroons. The neighbourhood of the Welle river in the Congo State also appears to be associated with the parasite, though according to Manson (1908) *F. volvulus* is not found on the Congo river: further investigations will probably modify this statement. Practically all the West African Colonies have contributed cases of *F. volvulus*, and we find the disease to which it gives rise reported as existing in the Congo, the Cameroons, Dahomey, Gold Coast, Sierra Leone and lastly, Nigeria. Dr Leiper informs me that the parasite has also been found in Uganda, so that, for the present, *F. volvulus* appears to be limited in a peculiar manner to the equatorial zone of the 'Dark Continent.' This may be due to lack of observation and insufficient data, or to a curious distribution of the intermediate host. Possibly both causes are at work. Personally, I venture to think, that, if every little subcutaneous tumour occurring in the negroid inhabitants of tropical Africa were examined *ex corpore*, it would be found that this form of filariasis was not at all uncommon. It is hardly necessary to say that in races so addicted to glandular enlargements as the African races are, mere casual observation is useless; consequently the suspected tumours must be excised for purposes of examination and diagnosis.

The subjoined table shows the chief clinical features of the five cases that form the subject of this paper.

	Case I	Case II	Case III	Case IV	Case V
Age	23	36	45	12	10
Sex	M	M	M	M	F
Occupation	Prisoner formerly a farmer	Prisoner formerly a farmer	Prisoner formerly a farmer	School boy	Freed slave ward
Race	Gadi (Pagan)	Igbirra (Pagan)	Igbirra (Pagan)	Onitsha (Pagan)	Fulani (Mahomedan)
District	German Cameroons	Kabba N. Nigeria	Kabba N. Nigeria	Onitsha S. Nigeria	Tola Benue River
Characteristics of Tumour	Multiple chest wall and scalp	Multiple chest wall	Multiple chest wall	Single Chest wall	Multiple flank adherent to iliac crest, size of hen's egg
Blood exami- nation	<i>Filaria perstans</i> found in blood	(Day) Nil	(Day) Nil	(Day & night) Nil	(Day & night) Nil
Remarks	No inconvenience from tumours, similar cases said to be common in his village	No inconvenience or symptoms			A brother said to have similar tumours

*Pathological Effects of Filaria volvulus.*

One of the most interesting features in the natural history of *F. volvulus* is the formation of subcutaneous fibroid tumours which vary in size from a split pea to a hen's egg. Clinically they are somewhat suggestive of dermoid tumours at first sight, being freely moveable over the subjacent tissues, while they are more or less firmly attached to the overlying skin. In older tumours, however, there may be very little mobility, and in one of my cases the growth was tightly adherent to the iliac crest. Apparently these tumours give rise to no symptoms, local or general; also, they must be very chronic in character judging from what I have gathered from the subjects of the disease. In my experience suppuration never occurs and Fülleborn has come to the same conclusion. As regards the position of the tumours Fülleborn (1908), quoting Zupitza, states that no part of the body is exempt, and they have been noted in such regions as the scalp, the chest wall, and the gluteal parts.

It is interesting to note that the observations of Kulz (cited by Fülleborn, 1908) are in accordance with mine in determining the

neighbourhood of the floating ribs as the favourite site for these tumours. Enucleation is usually not difficult though care should be taken to remove the tumours intact. As a rule, the excised growth is found to consist of two or three tumours of varying sizes all closely invested with a strong dense fibrous capsule. On section the composition of these tumours soon becomes apparent, and the peculiar testicular feeling experienced on palpation is explained. An opaque grumous fluid exudes from the tumour which is now seen to be composed almost entirely of an inextricable meshwork of filarial worms. These would appear to be most closely interwoven in what might be called the cortex of the tumour, while usually there is a central cavity which is bridged in every direction by the more or less disengaged portions of male and female worms.

Attempts to tease out portions of the tumour are attended with great difficulty owing to the manner in which these fragile little nematodes lie imbedded in the fibrous stroma. This fibrous tissue is probably formed as a result of reaction against the presence of the worms; and the fact that the adult *F. volvulus* has a rough and transversely corded cuticle makes it easy to account for the irritation that is set up. Or, it may be that the fibrosis is the result of some special secretion or excretion on the part of the worm. The fluid found in these tumours seems to vary somewhat according to the age of the tumour. In young tumours the fluid is scanty in quantity, and of a semi-opaque milky appearance. In older and larger tumours, on the other hand, it becomes thicker and more opaque, and takes on a dirty yellowish tinge; it may even assume a reddish colour as Zupitza has pointed out (cited by Fülleborn, 1908). Microscopical examination reveals the fact that the fluid of these tumours contains ova and embryos in large quantities together with fatty particles and cellular debris. As regards the adult inhabitants there seems to be a preponderance of females over males, but anything like an accurate count is well nigh impossible. In all probability a tumour 1 inch long would contain at least four females and three males, and here I think an observation in Prout's paper (1901) calls for some criticism. This observer states that he only found one female in a tumour 1 inch long and, on piecing the bits together, it measured 16 inches. It seems very doubtful whether a worm so narrow and slender would attain such a length, and it appears probable that the pieces belonged to several females. The small size of the male, too, rather supports this view as it is usually about half the length of the female.

*The Anatomy of Filaria volvulus.*

The mature worms are delicate cylindrical organisms of a greyish white colour, and are always found in a coiled up condition. They possess a thick and rigid cuticle which is annulated for the greater part of the body and is only seen broken in a transverse direction. The male worm measures from 20—32 mm. in length, and .2 mm. in diameter at its widest part. The female is both longer and thicker than the male, about twice as long, and measures .3 mm. in its thickest part.

The alimentary system is represented anteriorly by a mouth that opens at the bottom of a very small cup-like depression having a cuticular lining continuous with the cuticle of the body. There are no circumoral papillae. The oesophagus is a stout tube possessing a cuticular lining and measuring .8 mm. in length. The rest of the alimentary canal is a thin walled straight and narrow tube filled with opaque matter, which seems to show that the animal feeds on organised tissues, and does not wholly depend on lymph.

The *male worm* is hair like in form and maintains the same diameter throughout the greater part of its length. A very characteristic feature of the male is the single spiral twist at the end of the tail which is seen in all specimens. With care the male worm can be disentangled completely from the tumour and we found that the shortest worm measured 20 mm. while the longest was 32 mm. The cuticle is very finely ringed and resistant. The tail measures 0.07 mm. and ends in a bulbous blunt portion.

Fülleborn (1908) states that the tip of the tail is inverted to make a gutter like depression, but it is probable that this sulcus is apparent only, and is produced as a result of the arrangement of the caudal papillae. At 0.07 mm. from the head, the body of the male worm narrows to form a slightly narrower neck which is 0.05 mm. in diameter; thereafter the diameter increases uniformly until about the middle of the body the greatest width of 2 mm. is reached. Two groups of paired papillae occur at the posterior end of the worm:—(a) those near the anogenital pore or cloaca, and (b) those near the tip of the tail.

Concerning the number and arrangement of these papillae there is much diversity of opinion among the various authors, but our conclusions are based upon an examination of several specimens and are as follows.

In group *A* there are two pairs of pre-anal, and two pairs of post-anal papillae, all lying close together and almost touching one another. Occasionally the third papilla (i.e. the one immediately posterior to the cloaca) is situated more internally than the other three pairs, thus giving the deceptive appearance of three pairs only. This apparently has misled Fülleborn since he concludes that there are only three pairs. Group *B* contains two pairs of papillae situated at the tip of the tail; one pair being subterminal and ventral, and the other pair terminal and lateral. Midway, however, between the tip of the tail and the cloaca we have noted another large papilla, situated somewhat on the left side of the middle line, but having no corresponding papilla on the right side. The characters of this papilla, including the presence in it of a nerve fibril, leave no room for doubt that it is a true papilla, and not an artifact. The spicules are two in number and unequal in shape and size. They have been well delineated by Fülleborn. The larger spicule measures .23 mm. in length and ends in a sharp fluted point: the smaller measures .08 mm. in length and ends in a club shaped knob.

*Filaria volvulus* ♂.

Table of comparative measurements.

Author	Length of worm	Length of large spicule	Length of small spicule	Tip of tail to cloaca	Greatest diameter	Diameter of head
Prout	30.25 mm.— 30.35 mm.	—	0.082 mm.	—	—	—
Braun	30—35 mm.	—	—	—	0.14 mm.	0.04 mm.
Fülleborn	—	0.166 mm.	0.08 mm.	0.07 mm.	—	0.048 mm.
Parsons	20—32 mm.	0.28 mm.	0.08 mm.	0.07 mm.	2 mm.	0.04 mm.

*Female.* The extraction of a whole female worm is a matter of the greatest difficulty, and we were unable to obtain a complete specimen. This difficulty has been also experienced by other workers. Though they give certain lengths, there is room for doubt as to the correctness of their figures. Thus Prout's measurement of 16 inches has already been noticed (p. 362); Leuckart's specimen measured 60—70 cm., while Védý (1906) gives 18 cm. as the length of his specimen. The longest portion of female worm that we were able to obtain measured four inches.

The greater part of the female worm has a uniform width of 0.3 mm. but the anterior end becomes narrow and whip like, differing very little



in size and measurement from the same part in the male. We were not successful in our attempts to obtain the tail extremity of a female. In the middle of the worm the cuticular markings are more sharply defined and wider apart than in the male. Fülleborn rather happily compares the appearance of these cuticular striae to that of the wooden bands or hoops round barrels. He also lays great stress on the fact that Prout's female specimen did not apparently possess the same pronounced cuticular thickenings that his own specimen showed, and is rather inclined to believe that his own specimens constitute a new species. This seems improbable in view of the fact that his description of the male worm differs but very little from Prout's description of the male. Towards the extremities these cuticular striae in the female become finer and more closely set, and near the vulva they almost cease to exist. At 0.65 mm. from the head the genital pore opens without any marked protuberance of its lips. The vagina passes directly backwards as a thin walled canal, but shows a slight retort shaped dilatation just before its narrow aperture. The uterine tubules have thin cuticular walls with curious spiral markings not unlike the spiral vessels of vegetable morphology. Embryos were found at various stages of development in these tubules:—at one point coiled within the egg-shell; elsewhere partially coiled and distending the egg-shell almost to rupture; while in terminal portions of the tubule they were quite stretched out. These latter, like the embryos found in the fluid of the tumours, possess no sheath, a point on which all observers

*Filaria volvulus* ♀.

Table of comparative measurements.

Author	* Length of worm	Greatest diameter	Diameter of head	Distance of genital pore from head
Leuckart	60—70 cm.	—	—	—
Labadie-Lagrave and Deguy	25 cm.	0.15 mm. (immature worm)	—	—
Védy	18 cm.	—	—	—
Prout	16 in. or 40 cm.	.36 mm.	0.04 mm.	—
Fülleborn	—	.33 mm.	At .005 from mouth = .065 mm.	.55 mm.
Parsons	4 in. or 10 cm.	0.3 mm.	—	0.65 mm.
Brumpt	—	—	—	0.760 mm.
Braun	60—70 cm.	0.36 mm.	0.04 mm.	—

\* The measurements here recorded relate to the length of portions only of the female worm: moreover many of the figures must be taken with considerable reserve.

seem to agree. The embryos of *F. volvulus*, however, have a much thicker cuticular integument than is seen in other microfilariae of man, and the cuticle is transversely striated.

The *Embryos* measure 24 mm. in length. In stained specimens we were able to make out the central core of nuclei as described in the microfilariae. These granules, however, appeared to be more numerous and smaller than usual and we were not able to determine definitely the 'breaks' in the core corresponding to the rudiment of the nerve ring, to the excretory pore, and genital stolon.

*Development.* All observers agree that these worms live in local dilations of lymphatics, and that most probably the filarial embryos pass from these into the blood stream and are transmitted by biting insects. No observer has however detected the embryos in the blood. My own experience confirms these observations. Although it was evident from an examination of the contents of the tumours that millions of embryos were being discharged at the time of removal of the tumour I failed to find in repeated examinations any sign of these in the blood.

The distribution of the disease, as at present known, suggests the existence of a riverine intermediary, but of the further development of the parasite nothing is at present known, and this remains a problem for the future.

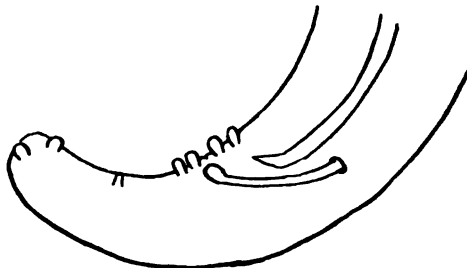


Fig. 1. *Filaria volvulus* Leuckart ♂.

Posterior end of a male specimen showing disposition of the papillae as seen from the left side. The solitary papillae lying midway between the peri-anal and the caudal groups is unpaired. The larger spicule is not shown in its entirety.

*Symptoms and Treatment.* As already stated, the presence of *F. volvulus* in the human subject appears to give rise to no symptoms or even inconvenience. The subjects of these tumours regard them as harmless possessions, and usually dismiss the matter with the remark that the tumour has existed for a very long time, and no longer con-

cerns them. To my knowledge pyrexia is unknown in connection with this form of filariasis. The only symptoms likely to occur are those caused by pressure. In one of my cases, I was asked to remove a tumour because it was unsightly and interfered with the proper disposition of the child's clothes. Treatment is simple and, if the 'disease' is merely local, effective. The tumours in themselves are easily enucleated with the aid of a little local anaesthesia: all the growth should be removed cleanly and intact.

#### CONCLUSIONS.

1. Time will probably show that *F. volvulus* is more common than has hitherto been supposed.

2. Analogy would lead us to suspect that *F. volvulus* is transmitted by some blood-sucking insect.

3. The geographical distribution of *F. volvulus*, as at present known, seems to correspond more or less with regions in Tropical Africa that are associated with such insects as have been proved to act as carrying agents in other parasitical affections.

4. While the adult male worm has been studied more or less completely, this does not hold for the female worm. The difficulty lies in the extraction of the worm from the tumour: the tail portion is nearly always left imbedded in the fibrous stroma and defies detection. What seems to be needed is some macerating medium in which the tumour would disintegrate without the worms becoming destroyed.

As regards preservatives a weak solution of Formalin proved very satisfactory, in the author's case, for preserving the tumours during transit.

5. Although the embryos of *F. volvulus* have not yet been found in the peripheral blood, it seems highly probable that some part of their existence must be spent in the general circulation.

Finally, I should like to acknowledge the kindness of the authorities at the London School of Tropical Medicine who allowed me to work in their excellent research laboratories. To Dr Leiper in particular I am much indebted, not only for the valuable help he so generously extended me, but also for his courtesy and friendly criticism.

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# THE SCHIZOGREGARINES: A REVIEW AND A NEW CLASSIFICATION.

By H. B. FANTHAM, D.Sc. Lond.,

*Christ's College, Cambridge, Assistant to the Quick Professor of Biology  
in the University; lately Assistant in the Zoological Department,  
University College, London.*

(Nine Figures and one Diagram.)

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## I. INTRODUCTION.

AT the present time, when so much interest is being displayed with regard to the study of the Protozoa, there is danger of too much attention being centred on those Protozoal organisms which give rise to disease in man and in the higher animals, to the neglect of many most interesting parasitic forms which do not necessarily give rise to disease,

and which consequently are not counted among the pathogenic Protozoa. The study of the life-histories of these relatively harmless organisms, in addition to being most interesting, may, however, throw light upon the biology of pathogenic forms. Among the former may be reckoned the Schizogregarines, a group of organisms of diverse external form, which are united by one special feature connected with their reproduction, namely, the intercalation in their life-cycle of an asexual method of multiplication. This feature serves to distinguish them from the common Gregarines, in which sexual reproduction alone obtains. The Schizogregarines have recently been the subject of several important papers by a small number of protozoologists.

The object of the present review is to draw wider attention to this very interesting group, and to put forward suggestions—more especially in relation to their classification. Having personally worked at the group, on the *Selenidiidae*, and being acquainted at first hand with *Siedleckia*, *Aggregata* and *Merogregarina*, I feel that the time has arrived when a review of the group will prove useful, especially since the literature relating thereto is both widely scattered and difficult to interpret.

A Glossary of Terms (see Appendix, p. 411) has been added for the general reader, at the request of the Editors.

## II. HISTORICAL SURVEY.

The term "Schizogregarine" dates from the year 1900, when it was introduced by Léger. Parasites belonging to the sub-order *Schizogregarinae* were known, however, before this date, for Aimé Schneider described the first of these forms, *Ophryocystis buetschlii* in a preliminary note (1883), and subsequently (1884) gave fuller details regarding it. The body of this parasite, which occurs in the Malpighian tubules of a beetle, is irregular in shape and possesses pseudopodium-like processes (Fig. 4). Schneider was doubtful as to the position which should be assigned to these parasites, and, on account of the somewhat peculiar feature just mentioned, which he considered diagnostic, he called them the *Amoebosporidia*. As such they are described in the works of Wasielewski (1896), and Labbé (1899) on the Sporozoa, being placed in the appendices<sup>1</sup> as a separate and problematic order

<sup>1</sup> The supposed parasite of cancer was once referred to the problematic group *Amoebosporidia* (see Minchin, 1903, p. 191) and that of variola and vaccinia was in 1895 placed in a genus *Amoebosporidium* by L. Pfeiffer.

of Sporozoa. A correct appreciation of the value of the amoeboid character of the organism in the two species of *Ophryocystis* (*O. buetschlii*, *O. francisci*) then known was first attained by Léger in 1900, when he described an allied parasite, which he called *Schizocystis gregarinoides*, from the gut of the larva of the fly, *Ceratopogon*. *Schizocystis* has a fixed and definite contour, and Léger showed that the so-called pseudopodia of *Ophryocystis* were merely stiff, root-like processes, for fixation of the extracellular parasite to the cells of the gut wall (Fig. 4, A, B).

In 1898 Caullery and Mesnil described a parasite, *Gonospora longissima*, in which an intracellular stage of asexual multiplication or schizogony occurred. In 1907 Brasil published his account of *Selenidium caulleryi* from the digestive tract of the Polychaete, *Protula tubularia*, and definitely placed the genus *Selenidium* (of the new family *Selenidiidae*) in the *Schizogregarinae*, pointing out that those forms of the parasite which divided asexually were intracellular in habitat, in contradistinction to the extracellular character of those of *Ophryocystis* and *Schizocystis*.

Léger (1907) at this time published a paper on the genus *Ophryocystis*. Recently there has appeared the paper by Léger and Duboscq (July, 1908) on *Aggregata* and "*Eucoccidium*," wherein the former (*Aggregata*, till recently considered as a gymnosporous Gregarine of crabs) is shown to be the schizogonic or asexual multiplicative cycle of a Schizogregarine, which passes through its spore-producing cycle in the gut-epithelium of cuttlefish (as the so-called "*Eucoccidium*" or *Benedenia*). Still more recently a detailed study of a new Schizogregarine has been completed by my friend and fellow worker, Miss Annie Porter (August, 1908)<sup>1</sup>. The parasite described by Miss Porter has been named *Merogregarina amaroucii*, and it occurs in the gut of a composite Ascidian from Australia. Other Schizogregarines belonging to the *Selenidiidae* have been described by Brasil and by the present author (1907) in various Annelids.

### III. OCCURRENCE OF THE VARIOUS GENERA.

The Schizogregarines almost invariably occur in the lumen of the gut of their hosts or in a channel leading therefrom, such as the Malpighian tubules in Insects. So far as present knowledge goes they

<sup>1</sup> The paper is now in the course of publication; a preliminary communication was published in Oct. 1908.

are more especially prevalent in the two great phyla, the *Arthropoda* and the *Annelida*. They are also known in *Mollusca* and *Asciidiacea*.

The genera *Ophryocystis* and *Schizocystis* occur among the *Insecta*. Various species of *Ophryocystis* are found in the Malpighian tubules of beetles belonging to the genera *Blaps*, *Akis*, and *Olocrates*, whilst *Schizocystis*, of which only one species, *S. gregarinoides*, is known, is found in the gut of the larva of a species of *Ceratopogon*, a dipterous Insect occurring in the Alpine Lake Luitel.

Various species of *Selenidium* have been recorded from Polychaetes: *Protula*, *Spio*, *Scololepis*, *Serpula*, *Dodecaceria*, etc. *Selenidiidae* were described from the Gephyrean *Phascolosoma* in 1907, and last spring (1908), while working at Banyuls, I observed two forms of Selenidiid parasites in the gut of the Terebellid, *Polycirrus aurantiacus*. Of the species of *Selenidium* from the above-mentioned Polychaetes many require re-investigation, for some of them were described many years ago by Ray Lankester (1863), Giard (1884) and others, the description often applying only to what is now known as the trophozoite phase, for *Selenidium* is characterised by the presence of well-marked longitudinal myonemes.

*Merogregarina amaroucii* Porter (1908) in the alimentary tract of the composite Ascidian, *Amaroucium* sp. (from Port Jackson, New South Wales), is the only example known up to the present of a Schizogregarine from a Protochordate. No true Schizogregarine has yet been recorded from the Chordates.

A number of species of *Aggregata* have been described recently by Léger and Duboscq as "coelomic" Gregarines which occur in certain crabs of the genera *Portunus*, *Eupagurus*, *Inachus*, etc. The sporogonic phases of the life-histories of these parasites have now been shown by Moroff, and Léger and Duboscq to occur in the Cephalopod molluscs, *Sepia* and *Octopus*. Moroff (1908) has described a number of new species from the octopus.

The stages of the organism formerly known as the gymnosporous Gregarine, *Aggregata* (Frenzel and Labbé), are the schizogonic stages of a parasite, whose sporogonic stages were formerly known as *Eucoccidium* or *Benedenia* or *Légerina* in Cephalopods. As the distribution of the Schizogregarines appears to be somewhat scattered, I have, for the sake of clearness and brevity, placed the main facts regarding them in tabular form (see p. 397 *et seq.*).

The effect of Schizogregarines on their hosts is chiefly to cause the



destruction of epithelial cells of the digestive tract; it is probably not of a serious nature.

#### IV. GENERAL MORPHOLOGY.

The youngest stage in the life-cycle of the Schizogregarine is that of the *sporozoite* (Figs. 1, 3, I) a minute, protoplasmic body with a distinct nucleus. It is usually somewhat sickleshaped, and measures  $5\mu$  to  $12\mu$  in length. As it does not differ markedly from the sporozoite of other Gregarines (collectively known nowadays as Eugregarines) its detailed structure need not be further considered. The sporozoite attaches itself by its rostrum or pointed end to an epithelial cell of the gut or lining of the Malpighian tubule, and grows. During this period of growth the parasite absorbs nutriment from its host, and it is known as a *trophozoite* (Minchin, 1903, p. 156). The shape of the trophozoite varies, and as in *Ophryocystis*, and to a less extent in *Schizocystis*, it is quite different from that of the other Schizogregarines and nearly all Eugregarines, it will be well to consider separately the trophozoite of each genus.

##### *Ophryocystis.*

The sporozoite in *Ophryocystis* grows, becomes pyriform and applies itself to the surface of the epithelium (Fig. 1, II). It then sends out stiff processes which serve to attach the parasite to the epithelium of the Malpighian tubules of the Coleopteran host. The trophozoite becomes somewhat conical in shape, and, while growing, its primitive single nucleus divides, so that it becomes multinucleate (Fig. 1, III). It is now known as a *schizont*, for cytoplasm gathers around each daughter nucleus and then the whole schizont (Fig. 1, IV) divides into small, uninucleate, somewhat pyriform masses (Fig. 1, V) termed *merozoites* ("schizozoites" of Léger). These migrate into the lumen of the tubule, and later attach themselves between new host cells by means of their processes, and so start a new infection in the same host. The multinucleate schizonts, which divide by multiple fission, are termed by Léger "mycetoid schizonts" (Fig. 1, III), to distinguish them from another form of schizont to be noted presently. The "mycetoid schizonts" somewhat recall the trophozoites of *Myxosporidia* in general appearance, but the resemblance is merely superficial. There is probably no close genetic relationship between these "mycetoid schizonts" and the *Myxosporidia*.

Schizogony continues only for a limited period, the merozoites growing into new schizonts. Towards the end of this period, when the host begins to react upon the parasites, the schizonts grow but exhibit only a few nuclei, some two to four (Fig. 1, VI, VII). These

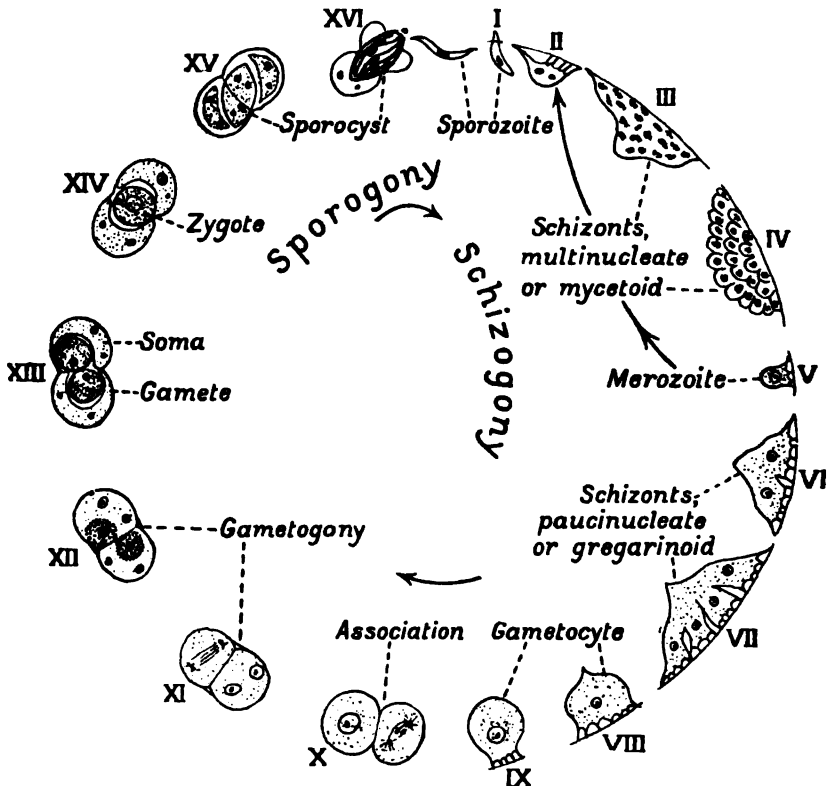


Fig. 1. Diagram of the life-cycle of *Ophryocystis* (based on that of *O. hessei*), after Léger (1907). Terminology altered. II–V, schizogony. VI–XIII, gametogony. XIV–XVI, sporogony.

"paucinucleate" forms, the "gregarinoid schizonts" of Léger, finally break up into *gametocytes* or sexual individuals ("gamontes" of Léger). Each gametocyte (Fig. 1, VIII) possesses only a single nucleus, and its growth goes on without further division until the parasite assumes a globular form (Fig. 1, IX) and becomes detached from the epithelium. It will be noticed that the growth of all the schizonts is *extracellular* and not within the living epithelium of the Malpighian tubule. This

does not occur in the *Selenidiidae*, and it is a point of interest and importance.

The gametocytes of *Ophryocystis* associate in pairs (Fig. 1, x). Nuclear division takes place (Fig. 1, xi), then nuclear reduction, and the two gametes (Fig. 1, xii, xiii) so formed, copulate (Fig. 1, xiv) and produce a zygote, which becomes a single octozoic spore (Fig. 1, xv, xvi). The details and significance of these stages will be considered later (see pp. 381, 382 and Fig. 4, D).

#### *Schizocystis.*

The trophozoite of *Schizocystis gregarinoides* Léger (1900) also needs special mention. It is stated to be of large size, some  $150\mu$  long, elongate, cylindrical in shape, with a hyaline, anterior portion, the whole being non-septate or monocystid. It is multinucleate, and the ectoplasm possesses a longitudinally striated cuticle, with a yellowish endoplasm containing refringent granules and rod-like bodies. The trophozoites are attached by anterior sucker-like ends to depressions in the wall of the intestine of the host. The number of nuclei in the trophozoite, which is really a schizont, may be as many as sixty, and the number increases with the size of the schizont, which starts by being a uninucleate, hyaline sporozoite. The schizont divides into a number of merozoites, some of which may be from  $20\mu$  to  $25\mu$  long, each with a single nucleus. The merozoites form uninucleate trophozoites of the second generation which are the gametocytes. Association and then encystment take place, and conjugation occurs between the gametes. In this way many octozoic spores are produced, after the manner common to the Gregarines.

#### *Selenidium.*

As a type of the remaining forms of Schizogregarines with *intracellular* schizogony, that of *Selenidium* may be considered. Species of *Selenidium* inhabit the digestive tract of various Annelids. One of the best known species is *Selenidium caulleryi*, the trophozoite and schizogonic stages of which have been fully described by Brasil (1907). This parasite occupies the digestive tract of *Protula tubularia*, and the infection is a heavy one. The trophozoite of *S. echinatum* (Fig. 2) from *Dodecaceria concharum* has also been well described by Caullery and Mesnil (1899).

The trophozoite of *Selenidium* is an elongate, vermiform, uninucleate organism (Fig. 2, A), roughly circular in transverse section, about  $75\mu$  long and  $25\mu$  broad in *S. caulleryi*. The anterior end is prolonged into a partly eversible or retractile epimerite, ectoplasmal in nature and clear and hyaline in appearance (Fig. 2, A, *ep*). The epimerite is lost later in the life of the organism. The posterior end is narrower than the anterior, the general shape being falciform, with a distinct curvature (Fig. 2, A). The trophozoite is motile, its movements being chiefly of flexion.

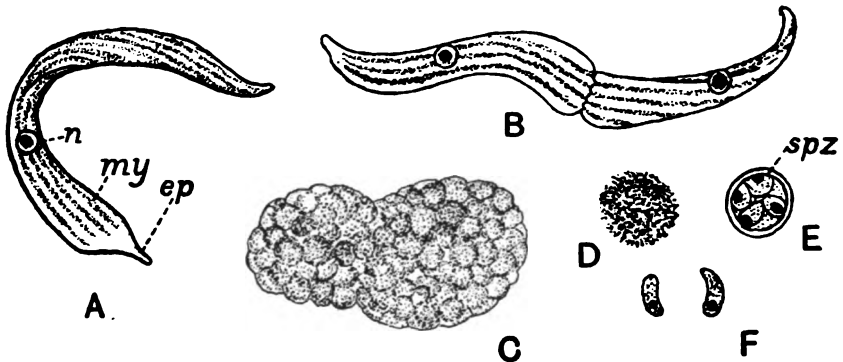


Fig. 2. *Selenidium echinatum*, after Caullery and Mesnil (1899).

- A. Free trophozoite, *ep* = epimerite, *n* = nucleus, *my* = myonemes.
- B. Two gametocytes in association.
- C. Cysts full of sporoblasts or young sporocysts, from a fresh preparation.
- D. Spherical sporocyst showing spiny exterior (epispor).
- E. Transverse section of sporocyst, with tetrazoic sporozoites, *spz*.
- F. Two free sporozoites.

The ectoplasm exhibits a series of fine, longitudinal, contractile striae, the myonemes (Fig. 2, A, *my*), which traverse the entire length of the body. Often 20 such myonemes are present, but the number varies for different species of *Selenidium* and may be smaller, *e.g.* four. The endoplasm is highly granular; little endoplasm is present in the epimerite region and it is less abundant at the posterior end of the body.

The nucleus is large and in a few species (*e.g.* *S. echinatum*) it is nearly spherical in shape (Fig. 2). In most species the nucleus is elongate oval, its long diameter being directed transversely with regard to the long axis of the animal (Fig. 3, II, vi). The nuclear membrane is very slightly marked, and the nucleus is filled with nuclear sap, somewhat granular in character, which surrounds the large laterally placed *karyosome*.

Intra-epithelial schizogony occurs in the *Selenidiidae*, and the intracellular stages are of long duration. The young schizonts in *S. caulleryi* resemble young trophozoites at first in being bluntly vermiform (Fig. 3, III), but they become more oval as growth proceeds. When they attain a length of about  $50\mu$ , nuclear fragmentation occurs (Fig. 3, IV), and the schizonts show 200 to 300 small, rounded nuclei scattered evenly through the cytoplasm, which does not seem to change. The cytoplasm next collects around each nuclear mass, and soon groups of small, curved, uninucleate merozoites are produced (Fig. 3, v). Each merozoite (in *S. caulleryi*) is  $10\mu$  to  $12\mu$  long and is motile.

The sporogony of *S. caulleryi* is not known. That of *S. echinatum* (from *Dodecaceria concharum*), as described by Caullery and Mesnil, (1899) may therefore be considered. In this case the two free

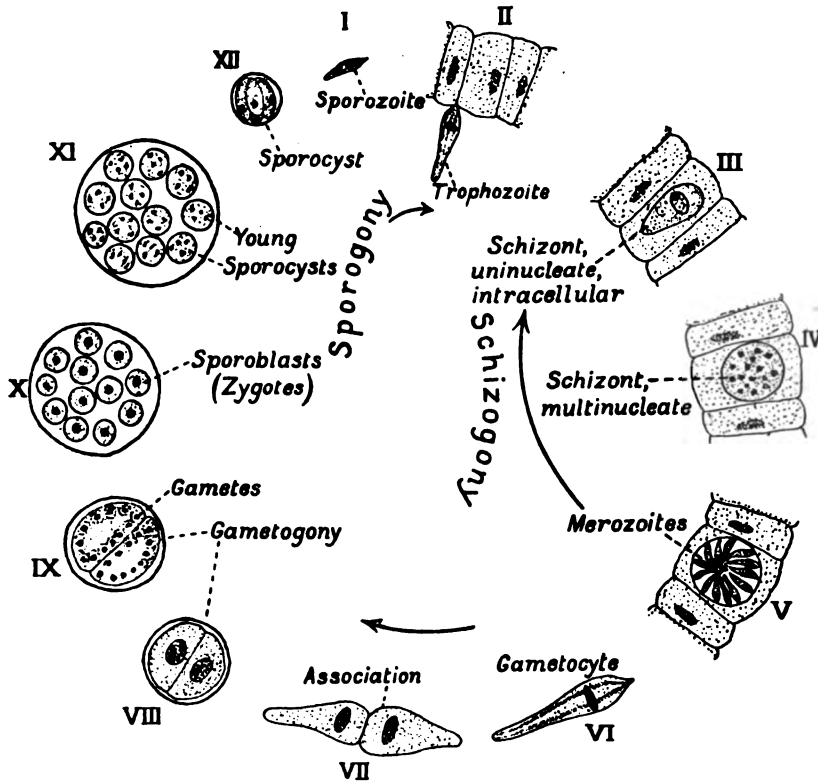


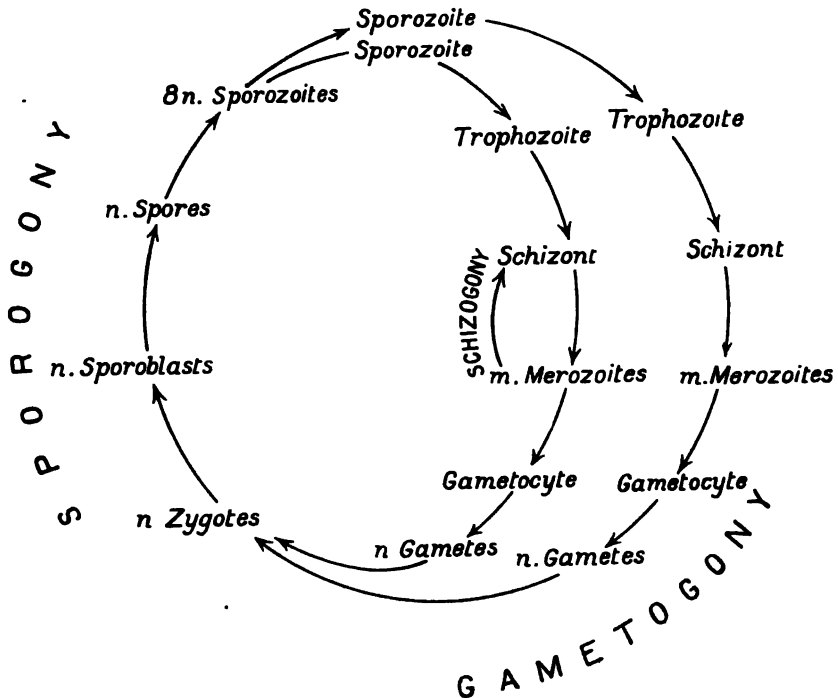
Fig. 8. Diagram of the life-cycle of *Selenidium*, based on that of *S. caulleryi* (Brasil) and *S. echinatum* (Caullery and Mesnil). Original diagram (H. B. F.). III—V, schizogony. VI—IX, gametogony. X—XII, sporogony.

trophozoites, which are at this stage gametocytes (Fig. 3, VII), associate at the ends corresponding to their epimerites (Fig. 2, B). Each gametocyte rounds itself off, and a common cyst is formed around them (Fig. 3, VIII). The nucleus of each gametocyte fragments and gamete nuclei are produced (Fig. 3, IX). Conjugation takes place between pairs of gametes and *sporoblasts* are produced (Fig. 3, X; also Fig. 2, C).

The sporoblasts give rise to spherical *sporocysts* (Fig. 2, D; Fig. 3, XI, XII) which are  $8\mu$  to  $10\mu$  in diameter. The cyst of the sporocyst in *S. echinatum* is finely spined (Fig. 2, D). Each sporocyst contains four symmetrically arranged sporozoites (Fig. 2, E; Fig. 3, XII). The small number of sporozoites in the sporocyst is most unusual among Gregarines, and, as Caullery and Mesnil point out, recalls the Coccidia. The spherical shape of the sporocyst is also unusual.

#### V. GENERAL LIFE-HISTORY.

The foregoing description and figures (Figs. 1, 3) will serve as outlines for the study of the Schizogregarine life-cycle, which I have set forth schematically in the following diagram.



In this diagram  $m$  refers to the variable number of merozoites formed from a schizont. Similarly,  $n$  refers to the large number of gametes formed from each gametocyte.

Each spore usually contains eight sporozoites.

The following points should be considered in connection with the above generalised diagram of the life-cycle:

Four sporozoites are present in *Selenidium* (e.g. *S. echinatum*), according to Caullery and Mesnil (see Fig. 2, E).

Intracellular schizogony prevails in *Selenidium* and *Merogregarina*.

Extracellular schizogony occurs in *Ophryocystis* and *Schizocystis*.

Nuclear reduction, so that one spore only is formed, takes place in *Ophryocystis*, this being expressed by the formula  $n = 1$  (see Fig. 4, D).

In the *Aggregatidae*, the schizogony takes place in one host (Crab), and the sporogony in another (Cephalopod Mollusc).

The number of sporozoites in the spore varies in the different species of *Aggregata* (e.g. 3, 4, 8, 16, 24).

## VI. DETAILED MORPHOLOGIES AND LIFE-HISTORIES.

The Schizogregarines at present comprise five well-marked families: *Ophryocystidae*, *Schizocystidae*, *Selenidiidae*, *Merogregarinidae* and *Aggregatidae*. The morphology and life-history of members of each of these families may now be set forth in some detail, avoiding, as far as possible, repetition of what has been written in the preceding section. It will be most convenient to consider these matters under the headings of the generic names, excepting in the case of the *Selenidiidae*.

### (1) Genus *Ophryocystis*.

Regarding the genus *Ophryocystis* Schneider, an account of the life-cycle of which has already been given, the following further points are of importance.

The presence of cytoplasmic processes or fixative filaments in the extracellular or trophic forms is not entirely without parallel in the Eugregarines, for in the genus *Pterocephalus* (Schneider), a Eugregarine from the digestive tract of *Scolopendra cingulata*, similar processes of a fixative nature are known, proceeding from the epimerite region of the trophozoite of *Pterocephalus* into the cells of the epithelium of the gut of the host. In *Ophryocystis* the various forms of trophozoite or

schizont are fixed by their anterior ends to the epithelial cells of the Malpighian tubules of the Coleopteran host.

As regards the action of these fixative processes and of the parasite generally on the host, Léger considers that hypertrophy of the cytoplasm of the host cells may ensue, or that atrophy may occur, following perhaps upon hypertrophy. Granules of pigment are often seen in the cells in the parasitised area.

Two forms of schizonts, first distinguished by Léger (1907), have been mentioned as occurring in *Ophryocystis*. These forms are: (i) **mycetoid schizonts**, "multinucleate" in character (Fig. 4, A), which may be of irregular form, containing many nuclei closely packed (as seen in *O. hessei*), and (ii) **gregaroid schizonts** with a well-defined contour and with fewer nuclei ("paucinucleate" of Léger), some 2 to 6 in number, as seen in *O. caulleryi* and *O. hessei* (Fig. 1, VI, VII).

The gregaroid schizonts were the only forms known until recently (1907). They are the more commonly occurring forms. Léger considers that these paucinucleate forms represent "la vraie forme grégارينienne de l'*Ophryocystis*." The gregaroid schizonts are the only ones which give rise to gametocytes ("gamontes" of Léger)<sup>1</sup> or sexual individuals (Fig. 4, B).

The mycetoid schizonts only give rise to merozoites ("schizozoites" of Léger) (Fig. 4, A), and so provide for the phases of schizogony. The daughter schizonts are formed either by (a) **plasmotomy**, wherein multinucleate portions or buds are constricted off, especially as in *O. hagenmuelleri*, which possesses branching schizonts, or (b) **schizogony**, as in the Coccidia, where the maternal cytoplasm breaks up and collects around each of the daughter nuclei, giving rise to uninucleate merozoites.

Mycetoid schizonts, at present, are only known to occur in the following species: *O. hessei*, *O. duboscqi*, *O. hagenmuelleri*, and possibly

<sup>1</sup> In describing *Ophryocystis* I have not used the terminology of Léger (1907), preferring to adhere to the older terminology, as used by Minchin (1908, p. 210), and introduced by Schaudinn more especially for the Coccidia. Léger used the term "gamonte" for a sexual individual or gametocyte. Although I admire Léger's work, and respect his unique knowledge of the Schizogregarines, the use of the term "gamonte" in this connection appears to me to be superfluous.

Léger's term "schizozoites," for the daughter forms resulting from schizogony (asexual multiplication or endogony) is obviously preferable to Schaudinn's term "merozoites." However, as the latter term is now well-established in the literature of the Parasitic Protozoa I have retained it. In view of the confusion already existing it appears undesirable to multiply technical terms. Wherever possible the terms in general use should be retained.



also in *O. francisci* and *O. buetschlii*, wherein schizonts containing about ten nuclei may be seen.

The phenomena of nuclear division in the gametocytes ("gamontes") during association (Fig. 4, C) are most interesting and significant. The single nucleus of the young globular gametocyte divides into two, giving rise to a "germinative nucleus" and a "somatic nucleus"

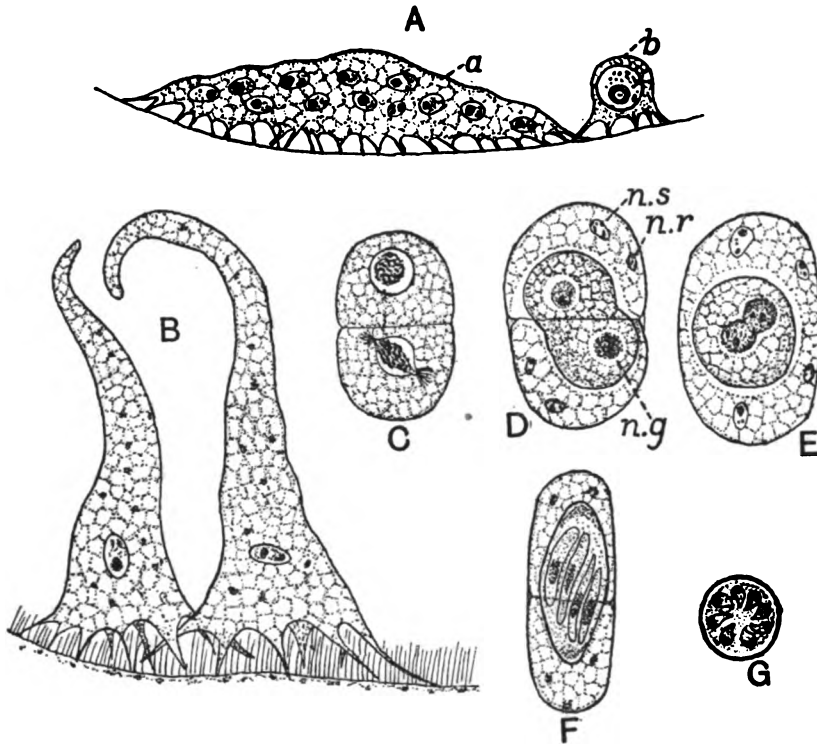


Fig. 4. *Ophryocystis*, various species, after Léger (1907).

- A. *O. hagenmuelleri*. Mycetoid schizont (a) at the beginning of schizogony. (b) Merozoite, already somewhat grown.
- B. *O. schneideri*. Gregarinoid gametocytes, with single nucleus, still attached to the epithelium of the host.
- C. *O. hessei*. Gametocytes in association.
- D. *O. mesnili*. Gametes in conjugation. *nr*=reduction nucleus, *ns*=somatic nucleus, *ng*=sexual nucleus (pronucleus).
- E. *O. mesnili*. Zygote, with pronuclei still distinct.
- F. *O. schneideri*. Cyst with ripe sporocyst. Falciform sporozoites represented inside.
- G. *O. schneideri*. Transverse section of sporocyst showing octozoic spores.

(Fig. 4, D, *ns*). The latter gradually degenerates. We have then formed in each gametocyte a somatic portion and a sexual or gametic portion. The germinative nucleus itself next undergoes division into two, which Léger considers to be a "reducing division." Two small nuclei are thus formed, respectively styled the "sexual nucleus" and the "reduction nucleus" (Fig. 4, D, *ng, nr*). The sexual nucleus is a pronucleus, *i.e.* the nucleus of the gamete proper. We have illustrated here an interesting case of the maturation of the gametes, with reduction and degeneration of all except one from each gametocyte.

Parthenogenesis, such as the origin of a sporocyst in each gametocyte of an associating pair, or the production of one sporocyst by one gametocyte the partner of which is sterile, is known to occur in *Ophryocystis*. Normally, copulation of two isogametes, as described in the foregoing section (p. 375), produces a single sporocyst. Inside the sporocyst eight sporozoites usually occur (Fig. 4, F, G).

## (2) Genus *Eleutheroschizon*.

*Eleutheroschizon duboscqi* Brasil (1906) is a parasite of the gut of *Scoloplos armiger* Oerst (= *Aricia muelleri* Rathke) and was first described by Brasil. It occurs fixed to the epithelium or free in the lumen of the gut. The fixed individuals are about  $30\mu$  long, and are dome- or bell-shaped. Their protoplasm is highly alveolar and their bases lie in hollows in the gut of the host. At the free extremity there is a cap with marked affinities for chromatin stains (Fig. 5, A, *chr*). The base is lobed, thus affording a superficial resemblance to *Ophryocystis*.

The schizogony only is well known. The merozoites are claviform and about  $2\mu$  to  $5\mu$  long. They glide between the cilia of the gut epithelium, and then penetrate for about half their length into the cells. They are never completely intracellular. Each merozoite grows to about  $8\mu$  in length. The part of it containing the large, vesicular nucleus with its prominent karyosome is internal, the rest external. When the organism is about  $10\mu$  long, an apical point appears, into which the nucleus migrates. The parasite may merely continue to grow and remain uninucleate or it may develop into a schizont (Fig. 5, B). The schizogony is extracellular.

When the parasite has attained the size of  $15\mu$  to  $20\mu$ , schizonts are formed, and nuclear division begins. A succession of such nuclear divisions occur, and the nuclei seem to be situated on bands of undulating protoplasm (Fig. 5, B). The nuclei finally reach the

periphery, and are extremely numerous and very small (Fig. 5, C). The protoplasm gathers around each nucleus, thus giving rise to a number of merozoites, irregularly arranged, and surrounding a mass of highly vacuolated, residual protoplasm (Fig. 5, D). Sporogony has never been seen, but it is probable that the uninucleate forms are gametes.

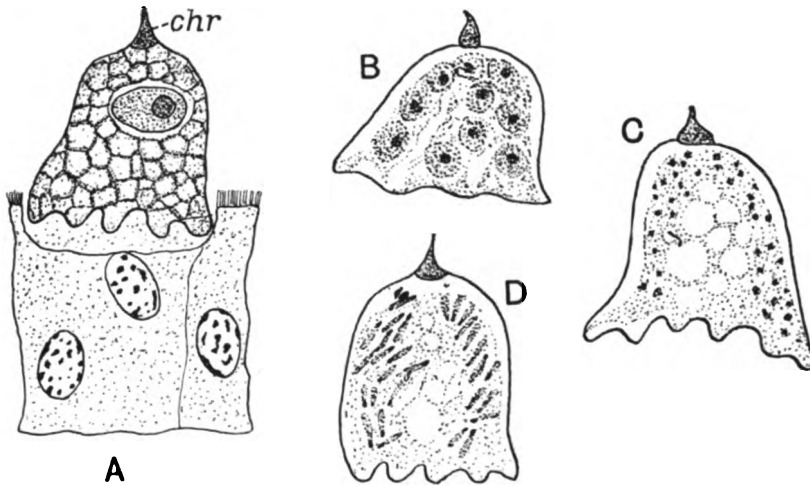


Fig. 5. *Eleutheroschizon duboscqi*, after Brasil (1906).

- A. Uninucleate schizont (trophozoite), fixed to the gut-epithelium of the host. chr=chromatic cap.
- B. Nucleus of schizont in process of division, the daughter nuclei situate on undulating cytoplasmic bands.
- C. Further stage in nuclear division. Protoplasmic bands more separated. Vacuoles present in residual protoplasm.
- D. Fully differentiated merozoites, lying near the periphery, away from the masses of highly vacuolated residual protoplasm.

### (3) Genus *Schizocystis*.

No figures have yet been published of *Schizocystis gregarinoides*, of which an account has been given on p. 375. The absence of figures is unfortunate, but L  ger, the discoverer of this interesting and unique organism, promises a paper thereon at an early date. Through the kindness of Professor L  ger I have been able to examine some sketches of *Schizocystis*. I was glad to note the likeness in body-form of the multinucleate trophozoite (schizont) to that of *Siedleckia* (Fig. 6). In each organism there is concurrent increase in the number of nuclei and in the size of the schizont. The merozoites, which are large,

claviform and uninucleate, grow without change of form into gametocytes. The gametocytes associate in pairs, encyst, and form isogamous gametes, which conjugate in pairs and give rise to many sporocysts, biconical in shape and peripherally arranged inside the gametocyst. The spore-formation therefore takes place on the plan generally prevailing among the Gregarines.

#### (4) Genus *Siedleckia*.

The genus *Siedleckia* Caullery and Mesnil (1898) was created for *Siedleckia nematoides*, a somewhat aberrant parasite inhabiting the digestive tract of the Polychaetes, *Scoloplos muelleri* and *Aricia latreillei*<sup>1</sup>. The discoverers have noted its resemblance to vermiform Schizogregarines like *Selenidium*. It is also very like *Schizocystis* in appearance, for it is nematoid in shape, and it is multinucleate. It has a similar habitat to *Selenidium*, and performs similar movements. The parasite, as stated, is a small vermicular organism, of which one extremity is fixed to the wall of the gut and is immobile, while the other or distal extremity is free, and executes vigorous helicoid movements of torsion and flexion.

The size of *Siedleckia* varies, specimens ranging in length from  $8\mu$  to  $150\mu$ . While they are very transparent, their protoplasm is granular. In life, clear areas are seen at intervals. These are the nuclei, which appear to greater advantage in stained preparations. The young forms are somewhat spindle-shaped and have few nuclei (Fig. 6, A); as they increase in size nuclear multiplication occurs (Fig. 6, B, C). The nuclei at first lie one behind the other in a single row (Fig. 6, B, C, D), but as they increase in numbers the order is broken and several rows may be seen (Fig. 6, E). This nuclear increase is far more noticeable in the distal or free end, which is rounded, than in the proximal portion (Fig. 6, E), which may be attached to the cells of the host. When the number of nuclei reaches about 120, asexual reproduction commences.

The parasite is now about  $150\mu$  long, and contains a relatively large number of nuclei. Its growth still continues, but at the same time protoplasm collects around certain peripheral nuclei at the distal end, and these become successively constricted off (Fig. 6, F) as small, spherical masses, each with a very small number of nuclei (Fig. 6, G). Each of these buds probably can develop into a new *Siedleckia*.

<sup>1</sup> Dobell (Q. J. M. S., Jan. 1909) found *Siedleckia* in *Aricia fatida*.

(Fig. 6, H). This method of reproduction, which is a form of schizogony, is comparable to the simple plasmotomy (Doflein) occurring in the *Neosporidia*. Up to the present this is the sole form of reproduction that has been described; the sporogony and mode of infection of the host are unknown. It is possible that resistant forms of *Siedleckia* exist, as Caullery and Mesnil state that they only obtained their material at a particular period of the year (August and September). Probably if the hosts were searched at their time of reproduction, the resistant phases of the parasite would be found.

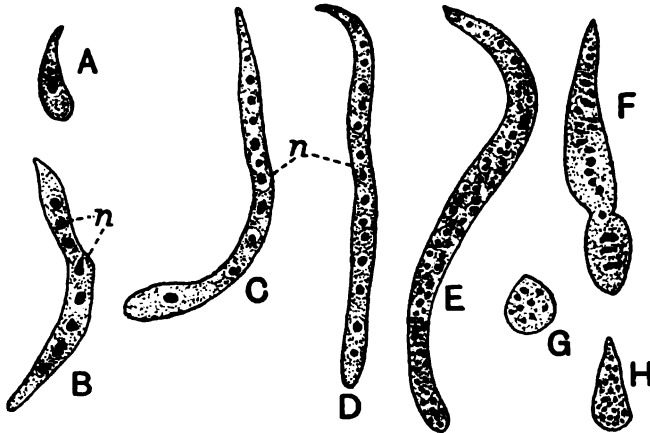


Fig. 6. *Siedleckia nematoides*, after Caullery and Mesnil (1899).

- A. Young trophozoite with two nuclei.
- B. Older form, with nuclei (n) in a single row.
- C, D. Trophozoites still older, nuclei in single file.
- E. Full-grown parasite. Nuclei in a single row at the more pointed end, in two or more rows at the distal or rounded end.
- F. Parasite showing small multinucleate portions constricting off, by plasmotomy.
- G. Young, somewhat spheroidal form, produced by constricting off the parent, as in F.
- H. Older stage of a form, such as shown in G, which is now growing into a vermiform trophozoite.

The systematic position of *Siedleckia* still remains undetermined. The general appearance and asexual multiplication by plasmotomy are certainly most suggestive of what has been described by Léger (1900) for *Schizocystis*, while certain of Léger's sketches (see p. 383) of *Schizocystis* further support the view that there is probably some affinity between *Siedleckia* and *Schizocystis*. Caullery and Mesnil (1899), who described *Siedleckia*, noted its superficial resemblance to the

*Selenidiidae*, while they also saw some analogy with *Amoebidium*. Inasmuch as the protozoal nature of the latter organism has now been refuted, while *Siedleckia* is undoubtedly a Protozoon, the comparison with *Amoebidium* cannot stand. On the whole, it would be well to place *Siedleckia* and *Schizocystis* in one group, as Brasil (1907) has done, a course that has been followed by Léger and Duboscq, uniting them on account of their similarity of habitat, their resemblance to one another when undergoing nuclear multiplication, and their method of formation of daughter forms containing one or more nuclei.

The multinucleate condition of the trophozoite, forming a so-called "plasmodial" stage, has led Caullery and Mesnil (1905) to discuss *Siedleckia* in their excellent paper on the *Haplosporidia*. Until something is known as to the existence or otherwise of sporogony, the definite systematic position of *Siedleckia* cannot be fixed. In the preparations which I have seen, a structure of the nature of an epimerite seems to exist, and this feature would accentuate the gregarine-like character of the parasite.

#### (5) Family *Selenidiidae*.

There is at present one well-defined genus in this family, viz. the genus *Selenidium*. This genus is, however, somewhat difficult to define from the point of view of a complete life-cycle. The species placed therein, by Labbé (1899) (under the name *Polyrhabdina*), are in some cases very doubtful members of the genus. The type species is *Selenidium pendula* Giard (1884), originally described as from the body cavity of *Nerine* sp. *S. pendula* possesses longitudinal myonemes. The habitat of this parasite as given by Giard is incorrect, for Caullery and Mesnil (1899) re-discovered it in the digestive tract of *Nerine cirratulus*. Selenids were undoubtedly seen earlier (e.g. Kölliker 1845), but were usually considered to be Nematode embryos.

Mingazzini (1891) described Selenid forms, since they possessed longitudinally disposed myonemes, under the generic names of *Polyrhabdina* and *Esarhabdina*, according to the large or small number of the myonemes present. In 1892 Léger described trophic phases of Gregarines, which he placed in a new genus *Platycystis*, possessing myonemes longitudinally arranged, in some cases with a spiral twist.

In 1898 Caullery and Mesnil contributed a very important paper on a Gregarine exhibiting a schizogonic phase in its life-cycle. The

host under investigation was the Polychaete worm *Dodecaceria concharum*, and it contained two parasites, one in the coelom known as *Gonospora longissima*, and the other in the gut, known as *Selenidium echinatum*. Caullery and Mesnil apportioned the schizogony, which occurred in the gut epithelium, to the former (*Gonospora*). Brasil (1907, pp. 389—393) discussed the matter at length, and considered that the schizonts really formed a part of the life-cycle of *S. echinatum*. Intra-epithelial schizogony also occurs in the *Selenidium* described by Caullery and Mesnil from the gut of *Scololepis fuliginosa*.

The most detailed account of schizogony in a *Selenidium* is that given by Brasil (1907) for *S. caulleryi*, from the gut of the Polychaete, *Protula tubularia*. Brasil's researches on *S. caulleryi* have been summarised in the preceding section (see pp. 376, 377, and Fig. 3, III, IV, v).

Further researches on the schizogony of Selenids were published by Brasil and Fantham (1907) who studied species found in the gut of Phascolosomes (*Phascolosoma vulgare* and *P. elongatum*). Two species of *Selenidiidae* occur in these Gephyreans; the species are differentiated at present by the number of longitudinal myonemes occurring in the respective trophozoites (Fig. 7). In the **first species** (*a*), which is rectangular in section, each face has only some two or three myonemes (Fig. 7, A, B) which are broad and very apparent. Two forms are recognised in this species, one with elongate trophozoites (Fig. 7, A), whose breadth is about one-fifteenth of its length, and which may reach  $350\mu$  in length, and the other, a shorter, stumpy form, whose breadth is about one-third of its length (Fig. 7, B). The nucleus of the elongate forms is nearly spherical (Fig. 7, A), that of the stumpy form is transversely ovoid (Fig. 7, B). Intermediate forms, however, occur between these elongate and stumpy types.

The **second species** (*b*) possesses many fine, longitudinal myonemes (Fig. 7, C, D), some 30 to 40 in number, while the body is circular in section. Elongate (Fig. 7, C) and stumpy (Fig. 7, D) types occur, but the differences in length between them are not so marked, and the relation of the breadth to the length is never less than one-eighth.

Schizogony occurs in the epithelium of the gut of the Phascolosomes. In the deeper parts of the gut epithelium, the schizonts form oval cysts which project slightly into the coelom. Each schizont gives rise to some 30 to 40 merozoites measuring about  $12\mu$  in length. Lateral association has been seen between the Selenids with fine myonemes (Fig. 7, E).

Dogiel (1907) has described a *Selenidium* from the gut of *Sipunculus nudus*, which he places, not in the genus *Selenidium*, but in that of *Schizocystis*, as *Schizocystis sipunculi*. Dogiel's parasite is very probably

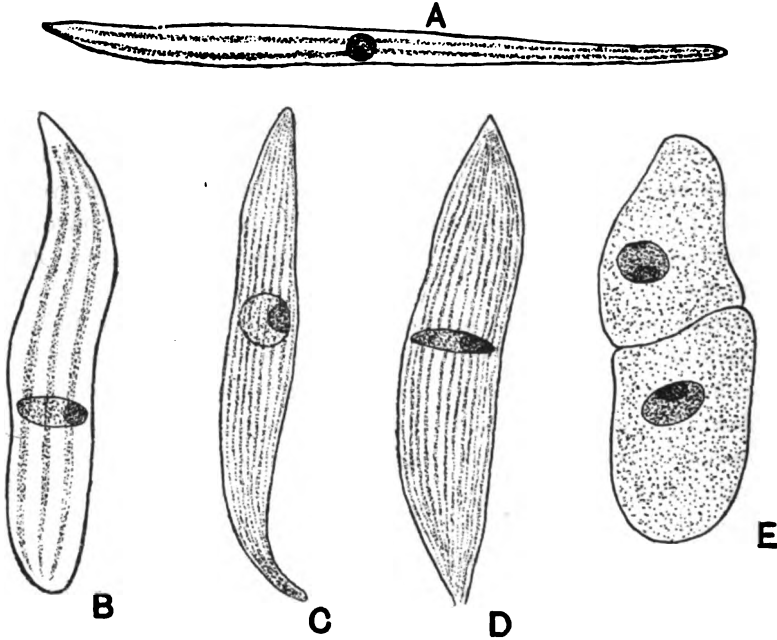


Fig. 7. *Selenidiidae*, described by Brasil and Fantham (1907), from the gut of Phascolosomes (*Phascolosoma vulgare* and *P. elongatum*). Original figures (H. B. F.), copied from drawings made with camera lucida.  $\times 600$  (except A which is  $\times 800$ ).

- A. Species ( $\alpha$ ), trophozoite, with few myonemes, elongate in form, and with rounded nucleus.
- B. Species ( $\alpha$ ), stumpy form, nucleus transversely ovoid.
- C. Species ( $\beta$ ), with many myonemes, slender trophozoite with rounded nucleus.
- D. Species ( $\beta$ ), stumpy form, with transversely ovoid nucleus.
- E. Species ( $\beta$ ), association of gametocytes.

a species of *Selenidium*, it is certainly a member of the family *Selenidiidae*, and is best termed, at present, *Selenidium sipunculi*. Dogiel's account of schizogony therein is apparently inaccurate (see Brasil and Fantham, 1907).

While working at Banyuls (Pyrénées Orientales) in the spring of this year (1908) I found similar Selenid parasites in the Terebellid, *Polycirrus aurantiacus*.



The shape of the epimerite in the trophozoite of *Selenidium* is variable. Caullery and Mesnil (1899) describe two forms of Selenid trophozoites with epimerites from *Cirratus cirratus*: (i) a comma-shaped variety (S. "en virgule"), and (ii) a semi-colon-like variety (S. "en point et virgule"). This variation is discussed by Brasil (1907), who attributes the truncated aspect to an invagination of the anterior pointed region (Brasil (1907), pp. 376, 384, 385).

Apart from the possibly injurious effect of the parasite upon the host cell, due to the organellae which serve for attachment, the investigations of Brasil (1907, Fig. 14) have shown that the merozoites of *S. caulleryi* may issue in masses into the lumen of the gut, thus displacing the epithelium.

It is interesting to note that species of *Selenidium* may in turn be parasitised. The Selenids of Phascolosomes (Brasil and Fantham, 1907) as well as certain species of *Selenidium* in Polychaetes (Caullery and Mesnil), and the *Platycystis* (= *Selenidium*) of Léger in *Audouinia* all contain minute parasites, one stage of which has the characteristic morula-form (cf. *Chytridiopsis* of Aimé Schneider).

In the following section an attempt has been made to set forth a complete list of the various species of *Selenidium* (see p. 399). This has entailed much labour, and the searching of many papers and figures. Of the lists of hosts of *Selenidium* given by Labbé (1899, who retains Mingazzini's name *Polyrhabdina*) and Minchin (1903), two members must certainly be radically revised as regards harbouring parasites belonging to the genus *Selenidium*. Regarding one of these, that described by Greeff (1885) as *Gregarina annulata* from the intestine of *Rhynchonerella fulgens*, it must be noted that it is transversely annulate as figured in the original, and seems to have been referred incorrectly to Mingazzini's genus *Polyrhabdina*. As at present understood, and pending further researches, Greeff's parasite has no place in the genus *Selenidium*. The other parasite, described by Ray Lankester (1866) as *Monocystis eunicae*, has been referred by Labbé to the so-called genus *Polyrhabdina*. It was found in the gut of *Eunice harassei*, and is listed by Minchin in the genus *Selenidium*. Reference to Lankester's figure does not support the view that "*Monocystis eunicae*" belongs to the genus *Selenidium*, and the discoverer makes no mention of longitudinal striations being present. Pending further researches it should certainly be removed from the genus *Selenidium*.

Most of the species at present placed in the genus *Selenidium* are only known in their trophozoite phase; further researches are greatly

needed on schizogony and sporogony. The sporogonic stages should be looked for especially at the time of reproduction of the Annelid hosts. Sections should be made of the digestive tracts of parasitised hosts for stages of schizogony of the parasite.

(6) Genus *Merogregarina*.

A new Schizogregarine from the alimentary tract of a Protochordate, namely, the Composite Ascidian, *Amaroucium* sp. has recently been described. The host came from New South Wales. The parasite belongs to a new genus, and Miss Annie Porter, who discovered it in 1908, has given it the name *Merogregarina amaroucii* (Fig. 8). The trophozoites occur in fair abundance in the gut of the host, and in some places a very heavy infection occurs in the lumen of the gut. The free trophozoites are  $23\mu$  to  $31\mu$  long and from  $11\mu$  to  $15\mu$  broad (Fig. 8, A, B). They are non-septate or monocystid. An epimerite about  $4\mu$  to  $6\mu$  long is present and it is shaped like a lance-head (Fig. 8, A, *ep*). Each trophozoite possesses a well-defined cuticle, a clear ectoplasm and a granular endoplasm. Myonemes are seen in the

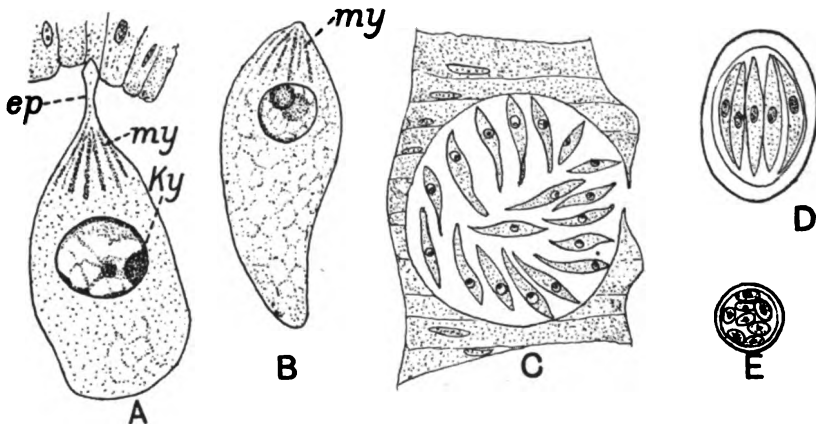


Fig. 8. *Merogregarina amaroucii* Porter (1908). Original drawings by Miss Annie Porter.

- A. Trophozoite with lance-shaped epimerite (*ep*). Myonemes (*my*) in pre-nuclear region. Large nucleus with marked karyosome (*ky*), and plasmosomes.
- B. Trophozoite whose epimerite is lost. Karyosome somewhat dumb-bell shaped.
- C. Section of gut of *Amaroucium* sp. showing group of merozoites cut longitudinally. The channel by which they issue singly into the lumen of the gut is here seen to be open.
- D. Sporocyst in longitudinal section, showing sporozoites.
- E. Sporocyst in transverse section, showing eight sporozoites cut across.

anterior region (Fig. 8, A, B, *my*), stretching from the epimerite to the neighbourhood of the nucleus in a fan-like manner. The nucleus is large and well-marked. It is vesicular, with a large karyosome (Fig. 8, A, *ky*) lying in a faint, chromatic reticulum. The karyosome may be spherical, notched or dumb-bell shaped (Fig. 8, B). One or more plasmosomes are frequently to be seen lying in the nuclear sap. There is a definite nuclear membrane with an irregular lining of chromatin internally.

The asexual cycle, or endogenous multiplication, is completely known. Schizonts occur within the gut epithelium of the Ascidian host. These schizonts are about  $17\mu$  long by  $10.5\mu$  broad. When they have attained this size the schizonts begin to sporulate. The nucleus of a schizont divides into 8 to 18 fragments of chromatin, there being no remains of the parent nucleus. The daughter nuclei are distributed fairly evenly throughout the mother cell (schizont). The cytoplasm of the latter collects around each daughter nucleus and so a small, but apparently inconstant, number of merozoites is formed (Fig. 8, C). These separate and find their way into the lumen of the gut and so account for auto-infection of the host.

Sporogony is also known, though somewhat incompletely. Two fully-formed trophozoites or gametocytes come together, associate and encyst. Finally sporocysts  $14\mu$  by  $11\mu$  are seen (Fig. 8, D). The sporocysts are oval and contain eight small sporozoites (Fig. 8, E) arranged "en barillet." These sporozoites serve for cross-infection.

*Merogregarina* reacts on its host, for trophozoites, while still in the gut lumen, are seen lying in bays or depressions in the epithelium of the gut. The effect of the schizont is, of course, more marked, and is of the usual character associated with intracellular schizonts.

*Merogregarina* is considered to be nearest the *Selenidiidae* in affinities, though it has octozoic spores, and the myonemes are only seen in the anterior region of the trophozoite.

This most interesting parasite, *M. amaroucii*, extends the distribution of the Schizogregarines to the *Protochordata*. It is also of interest to note that *Merogregarina* harbours a *Chytridiopsis*-like parasite within its protoplasm<sup>1</sup>. In this respect it resembles various *Selenidiidae*, as described by Brasil and Fantham, Caullery and Mesnil, and Léger (noted on p. 389).

<sup>1</sup> For further details see Porter (1908); a complete paper, fully illustrated, will appear in due course.

(7) Genus *Aggregata*.

The genus *Aggregata* was founded by Frenzel (1885) for Gregarines infesting various Decapod Crustacea (e.g. *Portunus arcuatus* and *Carcinus maenas*). These Gregarines in Crustacea had been seen by many of the earlier writers, e.g. by Cavolini (1787), Rudolphi (1819), and Diesing (1851). Frenzel considered that the coelomic cysts, really occurring in the peri-intestinal lymphoid tissue and projecting into the haemocoel of the crabs, belonged to the life-cycle of the Gregarines found in the lumen of the intestine of the same hosts, and that they arose from a conjugation. Labbé (1899) accepted Frenzel's genus and united it with *Porospora* of the lobster under the *Gregarina gymnosporica*, since naked sporozoites (so-called) occurred, grouped around residual protoplasm.

Geoffrey Smith (1905), however, showed that no conjugation occurred in the life-history of the "coelomic" Gregarine, *Aggregata inachi*, of *Inachus dorsettensis*. He found that at the beginning of sporulation there was only a single nucleus which divided and gave rise to daughter nuclei at the periphery of the cyst. These nuclei, each surrounded by a small mass of protoplasm, became so-called sporozoites. The intestinal Gregarines of crabs and the coelomic cysts of *Aggregata* must, then, be separated, as has now been done by Léger and Duboscq. No resistant sporocysts are formed by *Aggregata* in the crab.

Regarding the development of the sporozoites of *Aggregata*, Frenzel (1885) suggested the remarkable hypothesis that their further development might occur in Cephalopods, for which the crabs serve as food. This interesting forecast was found to be correct by Léger and Duboscq (1906), who demonstrated experimentally that the *Aggregata* of crabs and the *Eucoccidium* of Cephalopods only represent different stages in the life-cycle of one parasite. In this connection it is interesting to note that the gastric juice of *Sepia* is without action on the sporocysts of *Aggregata*. From the researches of Moroff and Léger and Duboscq it is now considered that we have in the parasite in question a *digenetic* and *heteroic* Gregarine, whose schizogony occurs as "*Aggregata*" in the crab, and whose sporogony occurs as "*Eucoccidium*" in the Cephalopod.

*Eucoccidium* was formerly unique among the *Coccidia* in possessing no schizogonous cycle, a sporogonic one only being known. The stages in the Cephalopods had been investigated by many well-known workers in the past, including Lieberkühn (1854—5), Eberth (1862), Schneider

(1875), Mingazzini (1892), Labbé (1896), Siedlecki (1898), and others. The work of Siedlecki is especially noteworthy. Species of *Eucoccidium* are known in various cuttlefish and *Octopus*. The multiplicity of species in Cephalopods, and of workers thereon, has led to great confusion in the nomenclature. The so-called Coccidian genus was variously known as *Eucoccidium*, *Klossia*, *Benedenia*, *Légeria*, and *Légerina*.

Léger and Duboscq have now ended the confusion by giving the name *Aggregata* (*Eucoccidium*) *eberthi* to the parasite whose schizogony occurs in *Portunus depurator* at Cette and in *Portunus arcuatus* at Roscoff, with its sporogony in *Sepia officinalis*. The methods of Léger and Duboscq, as before mentioned, were experimental. They fed specimens of *Portunus* on parasitised stomachs of *Sepia*. These stomachs contained sporocysts of *A. eberthi*. After a period varying from  $1\frac{1}{2}$  to 36 hours in the crab's stomach, the ripe sporocysts opened by the bursting apart of the two valves (Fig. 9, B) and the sporozoites were set free in the intestinal juice of the crab. The sporocysts of *A. eberthi* (Fig. 9, A), which are about  $9\mu$  in diameter, contain three sporozoites (Fig. 9, A, B). The free sporozoites are vermicular, curved or S-shaped (Fig. 9, B), and have a very slightly enlarged anterior extremity, with an elongate nucleus about  $5\mu$  long near the posterior end, without a distinct karyosome. The sporozoites average  $15\mu$  to  $18\mu$  in length by  $1.8\mu$  to  $2\mu$  in breadth. When set free the sporozoites rapidly penetrate the epithelial cells of the intestine of the crab. Most of them traverse the epithelium and soon attain the basal membrane, through which they try to pass. This basal membrane, however, is thick and resistant, and many of the sporozoites are stopped by it. Some succeed in piercing the basal membrane and gain the perintestinal lymphoid layer, where their further development takes place. The other sporozoites, which are unable to penetrate the basal membrane, remain imprisoned in the crab's intestinal epithelium, and ultimately die; they hypertrophy, become pyriform and degenerate.

The sporozoites (Fig. 9, C), which have succeeded in reaching the lymphoid tissue, remain there and continue their development. For a time their primitive length ( $15\mu$  to  $18\mu$ ) is retained, while they increase in breadth (from  $2\mu$  to  $8\mu$ ), becoming reniform and then nearly spherical (Fig. 9, D). The period of growth lasts about 10 days. During this growth in breadth, the nucleus changes in position, in shape and in structure. From elongate oval it gradually becomes spherical, and takes up a central position in the cytoplasm. The chromatin of the nucleus,

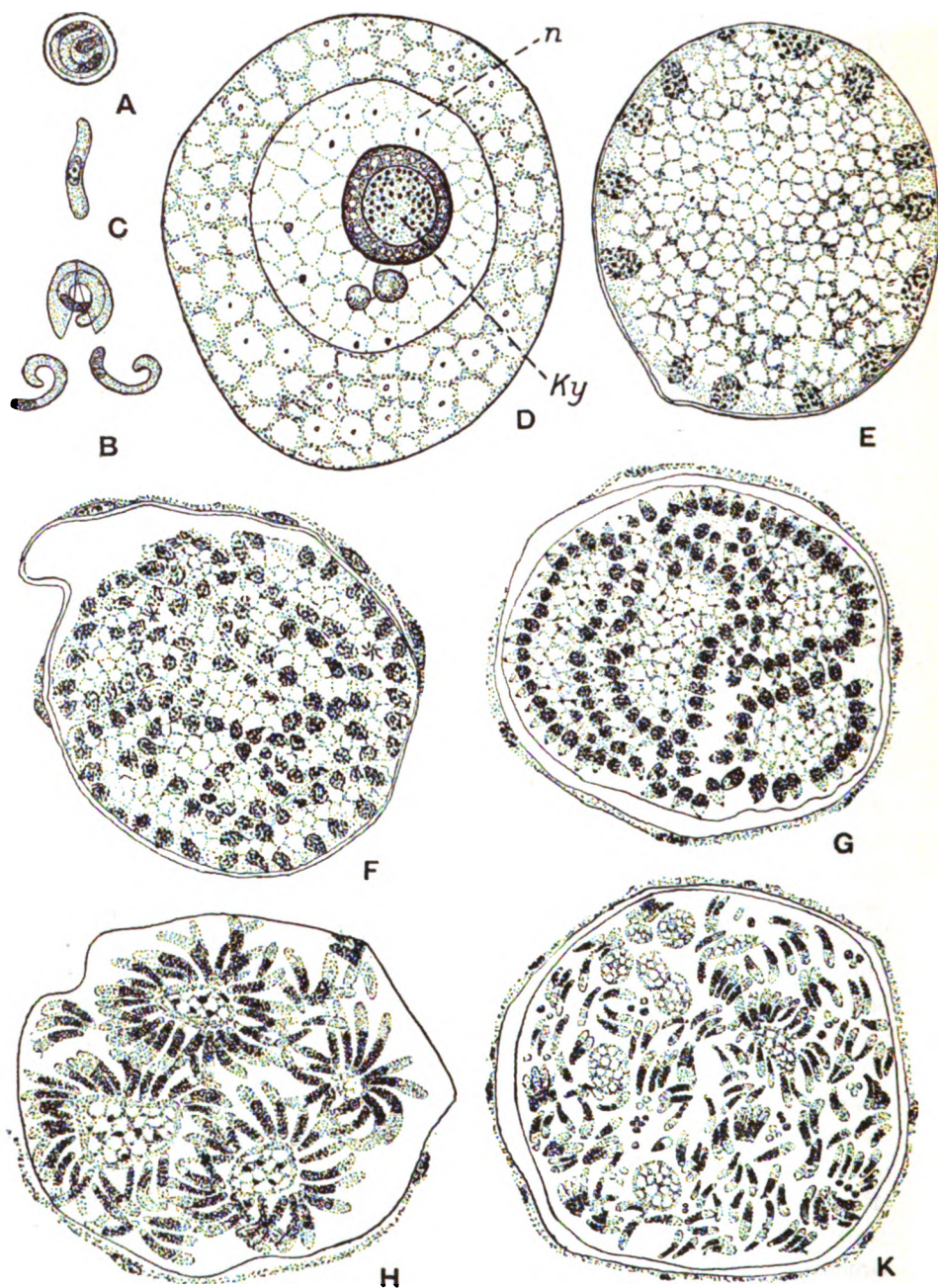


Fig. 9. *Aggregata eberthi* (Labbé). After Léger and Duboscq (1908).

- A. Sporocyst from the stomach of *Sepia officinalis*. Three sporozoites inside.
- B. Sporocyst showing valvular dehiscence, with two sporozoites free, the third still between the valves. Note posterior position of nucleus in the sporozoites. From gut of *Portunus*.
- C. Sporozoite at beginning of growth, after its passage through the gut-wall of *Portunus*. Nucleus has changed its position and become central.
- D. Further stage in growth; the parasite is now a young schizont. The nucleus (*n*) has become central, and the karyosome (*ky*) has attained its highest degree of complexity.
- E. Schizont. Nuclear division and migration of small masses of nuclear material to the periphery. This and following stages are enclosed in a thin cyst in the peri-intestinal lymphoid layer, projecting into the haemocoel of *Portunus*.
- F. Schizogony. Further nuclear multiplication.
- G. Schizogony. End of nuclear multiplication; nuclei arranged along serpentine strands of protoplasm. Commencement of differentiation of merozoites.
- H. Merozoites (schizozoites) fully differentiated and arranged at the periphery of somewhat rounded masses of residual protoplasm.
- K. Merozoites dispersed and lying free among masses of residual protoplasm.

at first arranged in the form of a reticulum, becomes peripheral and loses much of its capacity for staining. During this period a karyosome becomes evident (Fig. 9, D, *ky*) and gives off granules of chromatin into the cytoplasm. At this stage we have a young massive Gregarine provided with a spherical nucleus containing a large karyosome (Fig. 9, D), the organism being enclosed in a membranous cyst in the peri-intestinal lymphoid layer. The parasite is now a young schizont. The nucleus of the schizont, which possesses a nuclear membrane, complex karyosome and central reticulate zone (Fig. 9, D), then undergoes multiple division, giving rise to peripherally placed daughter nuclei (Fig. 9, E, F). These daughter nuclei are arranged along the edges of serpentiform, cytoplasmic islets produced by small invaginations of the mother cytoplasmic mass (Fig. 9, G). The daughter nuclei, with their cytoplasm collecting round them, give rise to rosettes of naked gymnosporos or merozoites arranged around spherical masses of residual protoplasm (Fig 9, H, K).

It is of interest to note that Léger and Duboscq found two forms of schizont. One form has a thick membrane and grows to about  $50\mu$  in length at the rate, roughly, of  $1\mu$  per diem, attaining their full size in 40 days. These are regarded as male Gregarines. The other form has a thin membrane and grows more rapidly and may attain a diameter of  $200\mu$  in 45 days, at the rate roughly of  $4\mu$  a day. This is regarded as a female Gregarine. The cytoplasm of a Gregarine which has practically attained its greatest size contains certain granules some of which have staining reactions like those of chromatin, while there are others, which,

though they do not stain in the same manner, are probably derivatives of chromatin. Grains of paramylum are also present, scattered in the network. Complicated nuclear changes take place during the growth both of the schizont and gametocyte.

There are slight differences in the merozoites formed from the two kinds of schizonts. The length of the merozoites averages  $10\mu$  to  $11\mu$ ; but slight differences occur in the breadth of the two varieties.

Léger and Duboscq remark that during the course of the development in the lymphoid tissue, a large number of young parasites are the prey of phagocytes which bring about their degeneration. This waste, together with the arrest of some of the sporozoites by the basal membrane, accounts for the rarity of cysts reaching maturity in nature.

Crabs do not feed directly on the stomachs of *Sepia* under natural conditions, but take up the sporocysts while eating the excrement of Cephalopods.

As regards the sporogonic stages of *Aggregata* in Cephalopods (which are initiated by merozoites from *Aggregata* of the crab), the most recent account is that of Moroff, who disagrees—especially in his preliminary communication in 1906—with the account given by Siedlecki in 1898. In his earlier papers Moroff has especially disputed Siedlecki's account of fertilisation. Moroff considers that the macrogametocyte forms more than one macrogamete, or that "fertilisation occurs at the time of formation of the [primary] sporoblasts," and that fertilisation occurs between anisogamous gametes, as in some Gregarines. However, Moroff (1908, p. 119) subsequently recognised that most of his earlier figures of fertilisation have nothing to do therewith, but merely represent stages in the division of the sporoblast. Moroff's grounds, then, for considering *Aggregata* as a Gregarine are insufficient (as remarked by Caullery in his review, *Bull. Inst. Pasteur*, vi. p. 723). New researches are needed on this important matter, and Siedlecki's descriptions of fertilisation are not yet refuted.

Moroff forms many new species of *Aggregata* from the parasites observed by him in an *Octopus* (sp?) of Cavalière (Var, Mediterranean). The specific differences according to Moroff are based on nuclear structure (e.g. variation of karyosome), or on the size of the gametocytes. Probably the number of species will ultimately have to be reduced. The sporocysts of species of *Aggregata* in the *Octopus* contain 8 to 24 sporozoites. Of the older species, *A. eberthi* and *A. octopiana* (Schneider) are the chief. Moroff's paper (1908) is largely written from the physiological point of view.



To summarise: it should be noted that the *Aggregatidae* form a perfectly distinct family, somewhat apart from other members of the *Schizogregarinae*. They differ (i) in the absence of association between their gametocytes, which entails subsequent fertilisation between anisogamous free gametes, and (ii) in a change of host being necessary to complete their life-cycle.

Léger and Duboscq (1908, p. 98) point out that in some Decapod Crustacea there occur both intestinal and coelomic Gregarines, e.g. in *Pinnotheres pisum* and *Eupagurus prideauxi*. In others there occur only intestinal Gregarines, as in *Chthamalus*, *Phronima*, *Gammarus*, *Athanas*, while in *Inachus dorsettensis*, G. Smith (1905) saw only coelomic Gregarines. These Gregarines, found in two entirely different situations, are quite distinct, a fact which was overlooked before the researches of G. Smith in 1905 and Léger and Duboscq (1907). For the intestinal Gregarines of Decapods, Léger and Duboscq (1907) proposed the generic name *Frenzelina*. The members of the genus *Frenzelina* are typical Eugregarines belonging to the family *Clepsydridinae*, and are entirely intestinal in habitat. The coelomic cysts found in the above-mentioned Decapods belong to *Aggregata*.

## VII. SYSTEMATIC.

In this section it will be convenient to define, as far as possible, the diagnostic characters of the various families of the Schizogregarines, and then to tabulate the genera and species contained therein, so far as known.

### 1. Family *Ophryocystidae*, Léger and Duboscq.

Schizonts extracellular, increasing simultaneously in volume and in number of nuclei.

Two genera: *Ophryocystis* and *Eleutheroschizon*.

#### (1) Genus *Ophryocystis*, A. Schneider.

Monocystid Schizogregarines with extracellular schizonts, conical in shape, fixed to the epithelium of the host by root-like cytoplasmic processes. Gametes isogamous. A single sporocyst, containing eight sporozoites, formed after conjugation.

Nine species are known from the Malpighian tubules of *Coleoptera* (especially *Tenebrionidae*).

Parasite	Habitat	Host	Remarks
<i>Ophryocystis buetschlii</i> , A. Schneider	Malpighian tubules	<i>Blaps</i> sp. (? <i>B. mucronata</i> )	
<i>O. francisci</i> , A. Schneider	" "	<i>Akis algeriana</i> , <i>A. acuminata</i>	Mycetoid schizont (?) known
<i>O. schneideri</i> , Léger and Hagenmüller	" "	<i>Blaps magica</i>	
<i>O. hagenmuelleri</i> , Léger	" "	<i>Olocrates gibbus</i>	Mycetoid schizonts known
<i>O. caulleryi</i> , Léger	" "	<i>Scaurus tristis</i>	
<i>O. mesnili</i> , Léger	" "	<i>Tenebrio molitor</i>	
<i>O. peresi</i> , Léger	" "	<i>Dendarus tristis</i>	
<i>O. hessei</i> , Léger	" "	<i>Omophlus brevicollis</i>	Mycetoid schizont known
<i>O. duboscqi</i> , Léger	" "	<i>Otiorynchus meridionalis</i> , <i>O. ligustici</i> , <i>O. fuscipes</i>	Mycetoid schizont known

(2) Genus *Eleutheroschizon*, Brasil.

Schizogony extracellular. Sexual reproduction unknown. The general form of the trophozoite (multinucleate schizont) and its manner of attachment to the intestinal epithelium of the host recalls *Ophryocystis*. The trophozoite possesses a chromatic cap.

One species only known at present.

Parasite	Habitat	Host	Remarks
<i>Eleutheroschizon duboscqi</i> , Brasil	Intestine	<i>Aricia muelleri</i> , Rathke = <i>Scoloplos armiger</i> , Ørst.	Schizogony only known

2. Family *Schizocystidae*, Léger and Duboscq.

Trophozoites cylindrical, elongate or vermiform, with an anterior clearer region. Monocystid. Schizogony extracellular, with nuclear multiplication during growth.

Two genera: *Schizocystis*, *Siedleckia*.

(1) Genus *Schizocystis*, Léger.

Trophozoites vermiform, affixed to the epithelium of the host by the anterior, clear extremity. Gametes isogamous, which, after conjugation, produce numerous octozoic spores.

One species only known.

Parasite	Habitat	Host	Remarks
<i>Schizocystis gregarinoides</i> , Léger	Intestine	<i>Ceratopogon</i> sp., larva	From Lake Lunel, Alps

(2) Genus *Siedleckia*, Caullery and Mesnil.

Trophozoites vermiform. Schizogony, by plasmotomy, only known. Sporogony unknown.

One species.

Parasite	Habitat	Host
<i>Siedleckia nematoides</i> , Caull. and Mesn.	Gut	<i>Scoloplos muelleri</i> , <i>Aricia latreilli</i> , <i>Aricia fatida</i> (Dobell)

## 3. Family Selenidiidae, Brasil.

Schizont intracellular, uninucleate during growth, becoming multinucleate at the end of its development. Trophozoites (gametocytes) free, vermiform, motile, with longitudinal myonemes.

One genus, *Selenidium*.

(1) Genus *Selenidium*, Giard.

This genus, as emended by Caullery and Mesnil in 1899, includes *Polyrhabdina*, Mingazzini (1891), *Esarhabdina*, Mingazzini (1891), and *Platycystis*, Léger (1892).

The epimerite may be slender and conical, or large and globular (Caullery and Mesnil, 1899). The latter form is probably due to invagination (Brasil, 1907). Spores spherical, spined and tetrazoic. Parasites of Annelids (Polychaetes and Gephyrea).

Many species, the majority of which are ill-defined, and need re-investigation, especially from the point of view of possible schizogonic stages.

The following list, which has entailed much labour, is the first of its kind for the genus; it is hoped that it is complete. It has been considered advisable to list the hosts in the first column, as so many of the species are unnamed. In view of our incomplete knowledge it appears inadvisable to supply names to such species of *Selenidium*.

Host	Habitat	Parasite	Remarks
<i>Aricia</i> sp.	Gut	<i>Selenidium sabellae</i> , Lank., 1863	
<i>Audouinia filigera</i>	,,	<i>S. cirratuli</i> , Lank., 1866	
<i>Audouinia tentaculata</i>	,,	<i>Selenidium</i> sp., C. & M.*, 1899	
<i>Cirratulus cirratus</i> (= <i>C. borealis</i> )	,,	<i>S. cirratuli</i> , Lank., 1866	Two species described by C. & M. (1899)
<i>Ctenodrilus serratus</i>	,,	<i>Selenidium</i> sp., C. & M., 1899	Small size
* C. & M. = Caullery and Mesnil.			

Host	Habitat	Parasite	Remarks
<i>Dodecaceria concharum</i>	Gut	<i>S. echinatum</i> , C. & M.*; 1899	Gametocyst and sporocysts known
<i>Nerine</i> sp.	„	<i>S. pendula</i> , Giard, 1884	Type species; coelomic habitat first given is incorrect
<i>Nerine cirratulus</i>	„	<i>S. pendula</i> in C. & M., 1899	C. & M. showed type-species is intestinal
<i>Phylodoce</i> sp.	„	<i>Selenidium</i> sp., Claparède, 1861	Original observation
<i>Polycirrus aurantiacus</i>	„	<i>Selenidium</i> spp., Fantham, 1908	
<i>Polydora coeca</i>	„	<i>Selenidium</i> sp., C. & M., 1899	Also said to occur in coelom, rarely
<i>Polydora flava</i>	„	<i>Selenidium</i> sp., C. & M., 1899	
<i>Pomatoceros triqueter</i>	„	<i>Selenidium</i> sp., C. & M., 1899	
<i>Polymnia nebulosa</i>	„	<i>S. costatum</i> , Siedlecki, 1908	
<i>Protula tubularia</i>	„	<i>Selenidium caulleryi</i> , Brasil, 1907	Schizogony described in detail
<i>Pygospio seticornis</i>	„	<i>Selenidium</i> sp., C. & M., 1899	Two species, one with single myoneme in which schizogony is known; the other with numerous myonemes
<i>Sabella</i> spp.	„	<i>S. sabellae</i> , Lank., 1868	
<i>Salmacina dysteri</i>	„	<i>Selenidium</i> sp., C. & M., 1905	
<i>Scolecopsis fuliginosa</i>	„	<i>Selenidium</i> spp., C. & M., 1899, 1901	
<i>Scolecopsis muelleri</i>	„	<i>Selenidium</i> sp., C. & M., 1899	
<i>Serpula contortuplicata</i>	„	<i>S. serpulae</i> , Lank., 1868	Notified by Léger as a <i>Platy-cystis</i>
<i>Spio fuliginosus</i>	„	<i>S. spionis</i> , Kölliker, 1845	
<i>Spio calcarea</i>	„	<i>S. spionis</i> , Léger, 1892	
<i>Spio martinensis</i>	„	<i>S. spionis</i> , Kölliker, 1845	The original account of schizogony is probably incorrect
<i>Sipunculus nudus</i>	„	<i>S. sipunculi</i> , Dogiel, 1907	
<i>Petalostoma minutum</i>	„	<i>Selenidium</i> sp., Brasil, 1907	Schizogony and encystment known
<i>Phascolosoma vulgare</i>	}	<i>Selenidiidae</i> 2 spp., Brasil & Fantham, 1907	
<i>Phascolosoma elongatum</i>			

\* C. & M. = Caullery and Mesnil.

#### 4. Family Merogregarinidae, Porter.

Trophozoite non-septate, ovoid, with a small, definite epimerite shaped like the head of a lance. Myonemes restricted to the anterior, pre-nuclear region. Schizogony intracellular. Sporogony known in part. Spores octozoic.

One genus.

##### (1) Genus *Merogregarina*, Porter.

Schizont intracellular, uninucleate during growth, becoming multinucleate at the end of its growth. Number of merozoites produced

from a schizont is relatively small. Gametocytes free, with anterior longitudinal myonemes.

One species only known at present.

Parasite	Habitat	Host	Remarks
<i>Merogregarina amaroucii</i> , Porter	Gut	<i>Amaroucium</i> sp.	From Port Jackson, New South Wales

### 5. Family *Aggregatidae*, Labbé.

Schizonts are sub-epithelial (coelomic), and their nuclear multiplication does not begin till growth is complete. Merozoites arranged around masses of residual protoplasm. No association between gametocytes, but copulation between free anisogamous gametes. Schizogony in Decapod Crustacea, sporogony in Cephalopod Mollusca.

One genus, containing several species.

#### (1) Genus *Aggregata*, Frenzel (= *Eucoccidium*, Lühe).

With the characters enumerated above. Many species differentiated by Moroff (1908), often on physiological grounds. These species, as given by Moroff, are tabulated below.

Parasite	Habitat	Host	Location	Gametocytes	No. of Sporozoites in Sporocyst
<i>Aggregata spinosa</i> , Moroff	Spiral caecum	<i>Octopus</i> [sp ?]	Cavalière (Var.), Mediterranean	♀ 250—300 $\mu$ ♂ 120—170 $\mu$	24
<i>A. légeri</i> , Moroff	„ „	<i>Octopus</i>	Cavalière	♀ 200—250 $\mu$ ♂ 120—170 $\mu$	16
<i>A. labbéi</i> , Moroff	„ „	<i>Octopus</i>	Cavalière		Sporocyst ?
<i>A. schneideri</i> , Moroff	„ „	<i>Octopus</i>	Cavalière		Sporocyst ?
<i>A. stedleckii</i> , Moroff	„ „	<i>Octopus</i>	Cavalière		16
<i>A. jacquemoti</i> , Moroff	„ „	<i>Octopus</i>	Cavalière	♀ 100—150 $\mu$ ♂ 80—110 $\mu$	16
<i>A. octopiana</i> , Schneider	Rectum	<i>Octopus vulgaris</i>		130—180 $\mu$	16
<i>A. duboscqi</i> , Moroff	Spiral caecum	<i>Octopus</i>	Luc-sur-mer	80—100 $\mu$	8
<i>A. reticulosa</i> , Moroff	„ „	<i>Octopus</i>	Cavalière	120—150 $\mu$	Sporocyst ?
<i>A. ovata</i> , Moroff	„ „	<i>Octopus</i>	Cavalière	200—300 $\mu$	Sporocyst ?
<i>A. stellata</i> , Moroff	„ „	<i>Octopus</i>	Cavalière		Sporocyst ?
<i>A. eberthi</i> , Labbé	„ „	<i>Sepia officinalis</i>	Cette; Trieste	90—120 $\mu$	8 (Schizogony in <i>Portunus arcuatus</i> and <i>P. depurator</i> )
<i>A. arcuata</i> , Moroff	„ „	<i>Sepia</i>	Cavalière	120—140 $\mu$	8
<i>A. mingazzini</i> , Moroff	Intestine	<i>Sepia</i>	Cette	120—150 $\mu$	4
<i>A. minima</i> , Moroff	?	?	Mediterranean	50 $\mu$	8 ?
<i>A. frenzelii</i> , Moroff	Spiral caecum	<i>Sepia</i>	Cette	80—100 $\mu$	Sporocyst ?
<i>A. mammillana</i> , Moroff	?	?	Cavalière	100—150 $\mu$	4

Of the following species, occurring in crabs, the schizogonic phases only are known.

Parasite	Habitat	Host
<i>Aggregata portunidarum</i> , Frenzel	Body cavity	<i>Portunus arcuatus</i> <i>Carcinus maenas</i>
<i>A. coelomica</i> , Léger	" "	<i>Pinnotheres pisum</i>
<i>A. vagans</i> , Léger and Duboseq	" "	<i>Eupagurus prideauxi</i>
<i>A. inachi</i> , G. Smith	" "	<i>Inachus dorsettensis</i> <i>Inachus scorpio</i>
<i>Aggregata</i> sp., Léger and Duboseq	" "	<i>Pachygrapsus marmoratus</i>

### VIII. CLASSIFICATION.

#### (a) Previous Classifications.

The origin of the name *Schizogregarinae* (Léger, 1900) has already been set forth in an earlier portion of this paper (p. 370). Minchin, in his article on the Sporozoa (1903), adhered to the classification as set forth by Léger, but included *Gonospora longissima* in the Eugregarinae, its original position. Caullery and Mesnil in 1898 had found merozoites in the gut of *Dodecaceria concharum*, a Polychaete which harboured the *Gonospora*, and Léger had suggested that *Gonospora* was possibly a Schizogregarine. Caullery and Mesnil associated the merozoites found by them with *Gonospora*, but in 1907 the question of the true adult, to which these merozoites belonged, was reopened by Brasil. The latter declared his belief that they were stages, not in the life-history of *Gonospora longissima*, but, rather, were part of the life-cycle of *Selenidium echinatum*, which also was a parasite of the gut of *Dodecaceria*. The question of the systematic position of *Gonospora* was incorporated by Brasil in an account of a new Selenidium, *S. caulleryi*, which he had discovered, and in his paper (1907) he advanced a new classification of the Schizogregarines. This was the first attempt to classify the Schizogregarines on a broad scale, and definitely introduced, in the *Selenidiidae*, forms with intraepithelial schizogony.

In the first family Brasil placed Schneider's genus *Ophryocystis*, and retained to some extent the historic name by styling the family the *Amoebosporidiidae*. *Schizocystis* he included with *Ophryocystis* in this family, in which he also placed *Eleutheroschizon*.

Brasil's second family was the *Selenidiidae*, marked by constancy of body-form and the presence of contractile myonemes on the body of the parasite.

The much discussed *Aggregata* (*Eucoccidium*) which sporulates in the gut wall of the cuttlefish and octopus, while its schizogony occurs in the crab, was placed by Brasil, on account of its widely different mode of life, in a separate (third) family, the *Aggregatidae*.

Quite recently, in July 1908, the paper of Léger and Duboscq on the *Aggregatidae* appeared, following on a long paper by Moroff. In this, Léger and Duboscq propounded a new classification of the Schizogregarines, based on the fact that in *Ophryocystis* two gametocytes give rise only to a single, octozoic spore. *Ophryocystis* is therefore placed in the subdivision *Monospora*, the remaining families being placed in the *Polyspora*, since, in the latter, two gametocytes associate, encyst and give rise to many gametes, each of which produces octozoic spores, as in the Eugregarines.

Léger and Duboscq's classification, including *Eleutheroschizon* and *Siedleckia*, appears thus in tabular form :

<i>Schizogregarinae</i>	<i>Monospora</i> .	<i>Ophryocystidae</i> ... <i>Ophryocystis</i> , <i>Eleutheroschizon</i> .
		<i>Schizocystidae</i> ... <i>Schizocystis</i> , <i>Siedleckia</i> .
	<i>Polyspora</i>	<i>Selenidiidae</i> ... <i>Selenidium</i> . <i>Aggregatidae</i> ... <i>Aggregata</i> .

In this classification the name *Amoebosporidia* is finally discarded, but unfortunately *Ophryocystis* and *Schizocystis*, which are alike in possessing extracellular schizogony, are widely separated.

The following important points in connection with Schizogregarines need to be considered in any scheme of classification :

(1) The extracellular character, as regards the tissues of the host, of *Ophryocystis* and *Schizocystis*.

(2) The intra-cellular character of the schizont in the *Selenidiidae* and the *Merogregarinidae*.

(3) The fact that the schizogony of the *Aggregata* occurs in a different host (Decapod Crustacean) from its sporogony, which takes place in a Cephalopod Mollusc. In the words of Léger and Duboscq, *Aggregata* is a Gregarine which is digenetic as regards phases of its life-cycle, and heteroïc as regards its hosts.

### (β) A New Classification.

We must not overlook the uncertainty which still prevails regarding the phenomena of fertilisation in the so-called *Eucoccidium*, as described by Moroff (1908) on the one hand, and Siedlecki (1898) on the other, and its bearing on the position of *Aggregata* (*Eucoccidium*). Taking

all the points, noted in the foregoing paragraph, into consideration, it seems to me that *Aggregata* stands apart from the rest of the group in being heteroïc. This fact is not obvious in Léger and Duboscq's classification, and the mode of sporulation, on which they base their classification, can be easily explained (see p. 382). The method of sporulation does not seem to me to be of so much importance as the heteroïc character of *Aggregata*, especially when it is remembered that the presence of extracellular schizogony brings *Ophryocystis* and *Schizocystis* into contiguity.

**Table showing the position of the Schizogregarinae in the Order Gregarinida.**

*Sub-order: Schizogregarinae*—Gregarines with a schizogonic phase in their life-cycle.

*Section I—Homoïca*—Schizogregarines whose complete life-cycle takes place in a single host.

*Sub-section (α) Ectoschiza*—With schizont extracellular.

*Ophryocystidae*, with a single sporocyst.

e.g. *Ophryocystis*.

(?) *Eleutheroschizon* (sporogony unknown).

*Schizocystidae*, with numerous sporocysts.

e.g. *Schizocystis*.

(?) *Siedleckia* (sporogony unknown).

*Sub-section (β) Endoschiza*—With schizont intracellular.

*Selenidiidae*, with longitudinal myonemes the whole length of the body.

e.g. *Selenidium*.

*Merogregarinidae*, with longitudinal myonemes confined to the anterior (pre-nuclear) region.

e.g. *Merogregarina*.

*Section II—Heteroïca*—Schizogregarines whose life-history is divided between two hosts, with schizogony in the one, sporogony in the other.

*Aggregatidae*, in crabs and cephalopods.

e.g. *Aggregata*.

The Schizogregarines may therefore be conveniently divided into forms whose life-cycle is completed in one host, i.e. homoïc forms, in contradistinction to the heteroïc *Aggregata*. Among the homoïc forms



we have those with extracellular schizogony, i.e. *ectoschizous* forms, and those with intracellular schizogony (e.g. *Selenidiidae* and *Merogregarinidae*) which are *endoschizous*. I would, then, divide the *Schizogregarinae* into two new sections, viz. the *Homoica* and *Heteroica*. The *Homoica* are divisible into two sub-sections, viz. the *Ectoschiza* and the *Endoschiza*. Including *Merogregarina* (Porter) this new classification is given in the accompanying table (p. 404).

#### IX. AFFINITIES.

The position of the Schizogregarines in the general scheme of classification of the Sporozoa is clearly within the order *Gregarinida*, and the sub-class *Telosporidia* (Schaudinn). Further, the Schizogregarines belong to the interesting assemblage of animals known as annectant forms. They link the Gregarines with the Coccidia, for in their trophic and sporogonic phases the *Schizogregarinae* resemble the *Eugregarinae*, while in the presence of an asexual multiplicative stage in their life-cycle they resemble the Coccidia.

Before concluding, the interesting and unique form *Schaudinnella henleae* (Nusbaum) may be mentioned for the light it sheds on the possible evolution of the *Telosporidia*. *Schaudinnella* possesses distinctly gregariniform, trophic phases, separate gametocytes without association or encystment stages, and well differentiated gametes, resembling in form those of Coccidia. The zygotes form sporozoites directly without the intervening formation of sporocysts.

A gregariniform *trophozoite* has two courses open to it, either to become a *schizont* and by schizogony or multiple fission produce a crop of merozoites, or to become a *gametocyte* and pass through a process of gametogony, giving rise to gametes. In the Gregarines two gametocytes associate and form a common cyst. Then each gametocyte divides to form many gametes which conjugate in pairs, producing many zygotes or sporoblasts, the whole sexual process having taken place inside a gametocyst. On the other hand, in the Coccidia many gametes are formed from the male (micro-) gametocyte, but only one gamete from the female (macro-) gametocyte, and fertilisation occurs between free gametes which encyst *after* the zygois. In *Schaudinnella* many microgametes are formed from each microgametocyte, but only a few (some 8 to 10) macrogametes from each macrogametocyte, and conjugation occurs between the free gametes. In the occurrence of conjugation between anisogamous, free gametes *Schaudinnella* more nearly resembles

a Coccidian, and is, indeed, intermediate in this feature between anisogamous Gregarines and the strict Coccidia. In most of the Schizogregarines the gametes are isogamous. *Schaudinnella* more nearly resembles the Coccidia in the particular characteristic (gamete formation) in which the majority of the Schizogregarines (leaving out *Aggregata* on which further information is required) differ from the Coccidia. *Schaudinnella* probably resembles a primitive type connecting the Gregarines and the Coccidia, devoid of schizogony (a differentiation evolved for the purpose of auto-infection of the host), yet already possessing the well differentiated gametes, characteristic of the Coccidia. From an ancestral, plastic form resembling *Schaudinnella*, the Eugregarines appear to have evolved on the one hand, and the Schizogregarines and Coccidia on the other; or perhaps, more correctly, the Schizogregarines (by acquiring schizogony) have evolved from the Eugregariniform type in the direction of the Coccidia. *Aggregata* would seem to link up the isogamous Schizogregarines and the anisogamous Coccidia.

Probably many other members of the Schizogregarines have yet to be discovered. It is possible that some of the Gregarines at present only known in the trophozoite phase may yet prove to have schizogonic stages, and their sporogony may occur at a strictly limited period of the year (see p. 390). There is here a wide field for research.

In conclusion it may be noted that the life-history of the Schizogregarines has a direct bearing on the advisability of retaining the separation of the Sporozoa into the *Telosporidia* and *Neosporidia* of Schaudinn, according as the reproductive phase of the life-cycle occurs at the end of or during the trophic or growing period.

The *Ophryocystidae* and *Schizocystidae* increase in volume during schizogony, and on this account would be placed in the *Neosporidia*, and not in the *Telosporidia* along with the *Eugregarinae*. Such a separation of the Schizogregarines and Eugregarines would be unfortunate, and scientifically unsound. Again, the *Microsporidia* are now placed among the *Neosporidia*, but they do not sporulate until they have completed their growth, which is a Telosporidian character. This is pointed out by Léger and Duboscq (1908, p. 101, footnote).

It seems preferable, then, to divide the Sporozoa, following Metchnikoff and Mesnil, into (a) *Ectospora*, wherein the spore-mother-cells or sporoblasts are formed at the periphery of the gametocyte, and (β) *Endospora*, in which the spore formation occurs in the interior of the body of the trophozoite, the spore-mother-cell or pansporoblast being separated off internally. The *Schizogregarinae* would then be

placed in the *Ectospora*, along with the *Eugregarinae*, the *Coccidiidea* and the *Haemosporidia*.

However, it does not seem profitable to discuss further the classification of the Sporozoa, on the basis of the life-cycle of the Schizogregarines. Classification is at the best only tentative, and must change with advancing knowledge. It is of much more importance to work out further *complete* life-cycles, and so—by filling in the gaps—to increase our knowledge of the facts which must underlie all classification.

#### X. SUMMARY.

1. The term *Schizogregarinae* Léger (1900) is the name now given to a sub-order of the *Gregarinida*, the remaining members of which are known as the *Eugregarinae*. The Schizogregarines were formerly known as *Amoebosporidia* Aimé Schneider (1884), a name given in misapprehension of the character of the cytoplasmic processes, fixative in function, present in the genus *Ophryocystis*. Two species of *Ophryocystis* (*O. buetschlii* and *O. francisci*) were the only members of this sub-order known before 1900.

2. At present the sub-order *Schizogregarinae* contains five families: *Ophryocystidae*, *Schizocystidae*, *Selenidiidae*, *Merogregarinidae*, and *Aggregatidae*.

3. All these organisms show well-marked schizogonic stages in their life-history, and—with the possible exception of the *Aggregatidae*—follow after the *Eugregarinae* in their methods of sporogony.

4. In *Ophryocystis* and *Schizocystis* the schizogony is extracellular, that is, these forms are *ectoschizous*. The life-cycle of the former is shown in Fig. 1. In these parasites the number of the nuclei in the schizont increases simultaneously with its volume.

5. In *Selenidium* and *Merogregarina* the schizogony is intracellular, in other words these forms are *endoschizous*. The life-cycle of the former is illustrated in Fig. 3. In these forms the schizont is uninucleate during its growth, only becoming multinucleate at the end of the growing period.

6. *Ophryocystis* forms only one sporocyst, a fact which has been emphasised by Léger and Duboscq (1908), by the placing of the *Ophryocystidae* in a special section, the *Monospora*. However, this apparent peculiarity is easily explained by a process of reduction and degeneration having taken place, affecting with one exception all the

gametes formed from each gametocyte. There is good morphological evidence in support of this explanation (see p. 382, and Fig. 4, D).

7. Figures of the interesting form *Schizocystis gregarinoides* (Léger, 1900) are not yet published, but a paper dealing with this organism is promised by Prof. Léger at an early date.

8. *Aggregata* differs from other Schizogregarines in that its schizogony takes place in one host (crab), while its sporogony occurs in another (Cephalopod mollusc). In this respect *Aggregata* resembles the *Haemosporidia*. The schizogonic phases in Crabs were formerly regarded as belonging to a gymnosporous Gregarine, *Aggregata* Frenzel, while the sporogonic phases were considered to belong to a Coccidian, *Eucoccidium* (*Benedenia*) in cuttlefishes and *Octopus*. Regarding this, Léger and Duboscq (1908, p. 102) write "...*Aggregata*, avec un changement de cycle coïncidant avec un changement d'hôte, c'est à dire qui soient à la fois digénétiques et hétéroïques."

9. It is evident therefore that the *Aggregatidae* stand apart. On this account, I suggest a division of the *Schizogregarinae* into two sections, termed respectively, the *Homoïca* (to include the first four families discussed in this paper, wherein schizogony and sporogony take place in the same host) and the *Heteroïca* (for the *Aggregatidae*).

10. Among the *Homoïca* we have extracellular schizogony (ectoschizous forms) in the *Ophryocystidae* and *Schizocystidae*, and intracellular schizogony (endoschizous forms) in the *Selenidiidae* and *Merogregarinidae*. This difference is not merely superficial, it requires to be emphasised, and for this reason I would divide the section *Homoïca* into two sub-sections, termed respectively *Ectoschiza* and *Endoschiza*.

11. The classification of the Schizogregarines, which I would propose, is as follows:

Schizogregarinae	{	Homoïca (nov. sect.)	{	Ectoschiza (nov. sub-sect.)	{	Ophryocystidae	{	Ophryocystis
						(?) Eleutheroschizon		
						Schizocystidae	{	Schizocystis
						(?) Siedleckia		
	{	Endoschiza (nov. sub-sect.)	{	Selenidiidae	{	Selenidium		
						Merogregarinidae	{	Merogregarina
{	{	Heteroïca (nov. sect.)	{	Aggregatidae	{	Aggregata		

12. Much further research is needed on the life-cycles of the *Endoschiza*, especially among the *Selenidiidae*, which occur so frequently in the *Annelida*. Sporogonic stages are at present unknown in *Eleutheroschizon* and *Siedleckia*.

13. In connection with the *Aggregatidae*, and to a less extent with the *Selenidiidae*, stress is laid upon the necessity of carefully distinguishing between "coelomic" and "gut" parasites. (See pp. 397 and 387.)

14. The *Schizogregarinae* form a most interesting link between the *Eugregarinae* and the *Coccidiidea*.

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## APPENDIX.

## GLOSSARY OF TERMS RELATING TO SCHIZOGREGARINES.

**Anisogametes.** Gametes showing sexual differentiation.

EXAMPLE : macro- and microgametes of *Aggregata*.

**Digenetic.** Having two phases in the life-cycle, viz. *schizogony* and *sporogony*.

**Ectoschizous.** Signifying that the *schizont* occurs on the *outside* of the cells of the host, and is attached thereto by cytoplasmic processes. In such cases the schizont is *extracellular*.

EXAMPLE : schizonts of *Ophryocystis*.

**Endoschizous.** Signifying that the schizont occurs *inside* the host-cell, that is, the schizont is *intracellular*.

EXAMPLE : schizonts of *Selenidium*.

**Endogenous reproduction.** The formation by the parasite of *merozoites* destined to re-infect the host. (See *Schizogony*.)

**Exogenous reproduction.** The formation by the parasite of resistant spores destined to infect fresh hosts. (See *Sporogony*.)

**Gametes.** Conjugating individuals developed from *gametocytes*, and giving rise to *zygotes*.

**Gametocyte.** The adult *trophozoite* matured for the production of *gametes*.

**Gametogony.** The process of gamete-formation.

**Gymnospore.** "Naked spores": not enclosed in a protective covering.

**Heteroic.** When two different hosts are required for the evolution of the complete life-cycle of a parasite.

EXAMPLE : *Aggregata*.

**Homoiic.** When the life-cycle of a parasite is completed within one host.

EXAMPLES : *Ophryocystis*, *Selenidium*.

**Isogametes.** Gametes which are morphologically similar.

EXAMPLE : *Ophryocystis*.

**Karyosome.** A nuclear corpuscle, containing a certain amount of chromatin in its substance, thereby differing from a nucleolus.

**Merozoite.** A free-moving uninucleate individual resulting from *schizogony*. A merozoite is often club-shaped.

**Plasmatomy.** The breaking-up of a multinucleate Protozoön into a number of portions or daughter-forms, each containing a variable number of nuclei.

EXAMPLE : *Siedleckia*.

**Schizont.** A full-grown *trophozoite* which has exhausted its host-cell and is about to multiply asexually, that is, divide directly into numerous uninucleate parts (*merozoites*).

**Schizogony.** A simple form of *sporulation* in which a *trophozoite*, without encysting, breaks up into numerous uninucleate masses of protoplasm termed *merozoites*. This process is sometimes called *asexual multiplication* or *endogenous reproduction*, and serves to increase the number of parasites in the host (auto-infection).

**Sporoblast.** A uninucleate mass of protoplasm arising from a *zygote*. A sporoblast gives rise to *sporozoites*.

**Sporocyst.** The tough chitinous membrane secreted on the outer surface of a *sporoblast*. A sporocyst enclosing eight sporozoites is often termed an *octosporic spore*.

**Sporogony.** The formation of resistant spores from a *zygote*, following upon a sexual act. This process is sometimes termed *exogenous reproduction*, and serves for the infection of new hosts (cross-infection).

**Sporozoite.** A fine, curved, falciform, naked mass of protoplasm formed from a *sporoblast*. The sporozoite is the agent which starts an infection.

**Sporulation.** A method of rapid multiplication by the formation of reproductive bodies (*spores*), each of which is a fragment of the parent body.

**Trophozoite.** An individual Sporozoön during its *trophic* phase, that is, during the time the parasite is absorbing nutriment from its host and is growing rapidly. The trophic period is one of "vegetative" growth.

A trophozoite may become either a *schizont* or a *gametocyte*.

**Zygote.** The individual resulting from the fusion of two *gametes*.



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